BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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VOLUME XXXIV.

WOODS HOLE, MASS.

JANUARY TO JUNE, 1918

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand
London, W. C.

AGENT FOR GERMANY
R. FRIEDLÄNDER & SOHN
Berlin N. W.
Carlstrasse, 11

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

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All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXIV. *January, 1918.* *No. 1.*

BIOLOGICAL BULLETIN

AUTOTOMY OF THE TAIL IN RODENTS.

F. B. SUMNER AND H. H. COLLINS.

It is perhaps not generally known that certain species of small rodents detach portions of the tail in a seemingly voluntary manner when they are seized by this member. Among the mouse-like rodents of California such an autotomy of the tail appears to be most typically exhibited by the pocket-mice (*Perognathus*).

It is the general practice of the writers, as probably of most breeders of mice, to pick up their animals by the tail. This appendage constitutes a convenient handle by which a mouse may be lifted, commonly relieving one of the risk of being bitten. But such a procedure is hazardous in the case of *Perognathus*. By a sudden gyratory movement of the body, the tail is likely to be severed at some point in its length, allowing the animal to make its escape and giving the pursuer something of a handicap in the race.

The whole performance is so suggestive of what occurs in many lizards that the question naturally arose in our minds: Can it be possible that autotomy, in the one case, as in the other, is followed by regeneration? This of course was not to be expected in a mammal, but the experiment seemed at least worth trying. In any case, the act and its consequences were thought to be worthy of a brief consideration.

The species upon which most of these observations were conducted was a rather large pocket-mouse, *Perognathus fallax fallax* Merriam, which is very common in the neighborhood of La Jolla. Despite the frequency of autotomy in this species, we soon found that all individuals cannot be depended upon to "perform" when desired. Those, in particular, which have been kept captive for some time seem to lose the tendency. More-

FIG. 1. *Perognathus fallax fallax*, showing normal condition of tail (somewhat reduced).

over, the act, when it does occur, is so sudden that one is commonly left wondering how it happened. But it was watched rather carefully in a number of instances, in order that the movements might be noted In some cases, the body seemed to undergo a rapid whirling motion, comparable with that of a rope tied at one end to a post and held by the hand at the other. The snout and tail, in this comparison, correspond to the points of attachment, the hind quarters of the body to the whirling central portion of the rope. A number of individuals which did not actually detach the tail were found to undergo less violent gyrations of the same sort. In other instances, however, there seemed to be an actual torsion of the appendage, resulting from a rotation of the body. In yet another, where the tail was held down upon a cement floor, the detachment was effected by a sudden leap.

In eight cases, in which the detached portions of the appendage were carefully examined, it was found that the fracture invariably occurred across one of the caudal vertebræ, commonly near its center. The tail is likely to be broken at almost any point in its length. The detached portion carries away with it a group of tendons of such length as to indicate that these have their points of origin within the body

itself. The possibility of a regeneration of the missing part of the tail was tested in fourteen specimens. Some of these underwent a voluntary autotomy, in the manner above described. In others, the breaking of the appendage was facilitated by the observer. These mice were all nearly mature, though probably most of them had not reached the limit of growth.

In the first seven specimens, the stump of the tail was measured shortly after the operation, but the length of the body was unfortunately not taken. Four of these specimens were living at the end of twelve months. In three cases, the tail has undergone a slight elongation (1–5 mm.), but this was doubtless merely an ordinary process of growth, accompanying the general growth of the body. In the fourth case, the recorded growth was 18 mm., though we believe this to be based upon an error in the first measurement. Except for the hair conditions, to be described below, there was no evidence of restoration of any part of the tail.

The next seven specimens were dealt with much more carefully. The length of the detached portion of the appendage was determined; likewise that of the stump, and the length of the body from the snout to the base of the tail.[1] Six of this second lot of mice were living at the end of nine months. When measured then, it was found that the tail stumps in five specimens had undergone a trivial increase in length, averaging about two millimeters. This was evidently incidental to a slight general growth of the body, since the mean body length, during the interval, had increased about four millimeters. In the sixth specimen, the tail was actually shorter, its condition indicating that it had been further damaged after the original operation.

Examination of the tissues of the tail in all of the foregoing specimens gave no suggestion of regenerative processes. In each case, the vertebral column terminated in a partial vertebra, this being evidently the one which was fractured in the process of autotomy.

There was, however, one very interesting result. In most of the specimens a dense tuft of elongated hairs had formed at the termination of the stump, the solid core of the latter being in

[1] In making the latter measurements the animal was etherized.

some instances enlarged at the end. This tuft was entirely a new growth, since the middle region of the tail is normally covered with very short hair. As the uninjured tail of this rodent terminates in a "pencil" or tuft of longer hairs (Fig. 1) the formation of such a tuft at the point of amputation gave the appearance of a restorative process. There are, however, considerable points of difference between the normal "pencil" and the secondary one. The former is produced by the gradual elongation of the hair which covers the tail, commencing with the posterior third or half of this member. The restored tuft (Fig. 2) commences much more abruptly, sometimes being

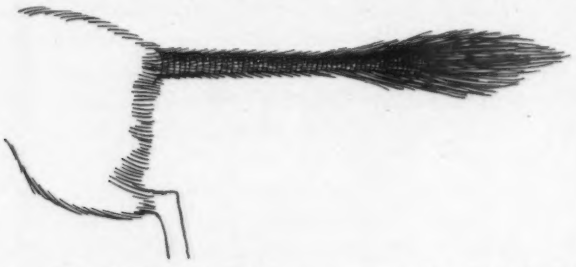


FIG. 2. Tail which has undergone autotomy about midway in its length, and upon which a new terminal tuft of hair has been produced.

confined to the end of the stump. Its hairs are much longer than those of the natural pencil, and their pigmentation is conspicuously decreased, being pale gray on the normally black dorsal side.

In two instances a small lateral tuft was likewise formed, not far from the end of the stump. In one of these cases, there was found to be an internal injury, corresponding in position to this lateral tuft.

The capture of two wild specimens with truncated tails indicates that this accident may not be uncommon in nature. There was, of course, no clue to the source of the injury. Both specimens had developed terminal tufts, one of these being unusually perfect.

The time required for the formation of a new "pencil" has not been definitely noted. We have record of its having arisen within two months, but we also know that more than four months may be necessary.

The writers offer no speculations as to the physico-chemical factors which bring about this excessive growth of the hair on the severed stump of the tail of *Perognathus*. A superficial analogy suggests itself between this terminal tuft and the cluster of adventitious shoots which arise from the stump of a tree. Possibly the analogy is more than superficial.

How general this process of autotomy is among the pocket-mice we can not say. We have noted its occurrence in only one other species, *P. panamintinus bangsi*, a small desert form in which the process is closely similar to that in *P. fallax*.

To what extent this phenomenon may be spoken of as a "protective adaptation," and whether or not it arose through natural selection, we are unwilling to conjecture. It is more than possible that a pursuing carnivore would sometimes be cheated of its prey in this manner, since the greatly elongated tail might readily be seized by the claws or teeth. The association between the fragile structure of this appendage and the peculiar instinctive responses of the animal is probably not accidental.

A phenomenon similar to that discussed above, but differing in essential features, has been observed by one of us in a species of *Peromyscus* (*P. boylei rowleyi*). This mouse likewise has a relatively long tail, and undergoes, when this member is seized, very much the same curious gyrations as does *Perognathus*. In the former species, however, the vertebræ are not broken, nor indeed is the central axis of the tail severed at all. The skin breaks at some point in its length, and slips off, leaving in one's hands the long tubular sheath which covered the appendage. This process is of such frequent occurrence as to be quite characteristic of these mice, which thus contrast strongly with the various other species of *Peromyscus* that we have observed. In the subspecies of *P. maniculatus*, for example, we have never observed the detachment of any portion of the tail or of the skin, in the course of handling several thousands of these animals. Nor have we observed it in any other genus of California Muridæ.

In *Peromyscus boylei rowleyi* we must again note what seems to be a significant correlation between structure and behavior. The skin of the tail breaks and slips off with remarkable facility.

Likewise, the animal, when seized by this member, makes what appear like vigorous and well-directed efforts to accomplish the severance. In *P. maniculatus*, on the other hand, the skin is far less easily detached, and the animal has scarcely ever been observed to undergo either bodily gyrations or torsion of the tail.

To what extent this detachment of the skin is to be regarded as an "adaptive mechanism," which has arisen because of its utility, we regard it as futile to discuss at present. A single observation may be cited, which, however, can not be regarded as throwing much light on the matter. One specimen of *P. boylei rowleyi* was caught by the tail in a spring mouse-trap, the body being uninjured. Under these conditions, the animal was unable to escape by slipping off the skin and died of shock or exposure. On the other hand, it must be stated that a considerable proportion of the living mice of this species which were lifted by the tail underwent this mutilation, in spite of considerable care being taken to prevent it. What happens to the exposed portion of the tail after the detachment of the skin, has not been observed. It probably dies and falls off.

SCRIPPS INSTITUTION FOR BIOLOGICAL RESEARCH,
August 8, 1917.

THE INTERRELATIONSHIP OF THE NUMBER OF STAMENS AND PISTILS IN THE FLOWERS OF *FICARIA*.

J. ARTHUR HARRIS.

I. Introductory Remarks.

A survey of the rapidly increasing literature must convince anyone that the problem of the factors which determine the sex of the organism is one of such complexity that it cannot be solved on the basis of any one kind of material or by any one method of research.

In the flowering plants the same individual may produce both eggs and sperm. The relative numbers of egg and sperm producing organs may vary from individual to individual, or from flower to flower within the individual.

It is reasonable to assume that definite genetic, morphogenetic or physiological factors underlie these variations. Any successful attempt to determine these factors and to measure their influence is just as truly a contribution to the wide problem of the physiology of sex as the more conventional breeding experiments and studies on the morphology of the germ cells.

The purpose of this paper is to point out certain hitherto unrecognized relationships between the number of sporophylls in the flower of the ranunculaceous genus *Ficaria*.

Heretofore those who have investigated the problem of the relationship between the number of stamens and pistils in the flower have been content to merely determine the correlation between the number of the two kinds of spore-bearing organs. Positive correlations of this kind should arise as the resultant of any sets of environmental factors which tend to increase both the number of stamens and the number of pistils in certain of the plants or individual flowers and to limit the number of both of these organs in others. Morphogenetically and physiologically it seems of far greater importance to inquire whether the relative

proportion of the two types of spore bearing organs is correlated with the total number of sporophylls, which in lieu of any better character may serve as a measure of the total influence of intrinsic and extrinsic factors influencing degree of development.

Several years ago Professor Pearson and I (Harris, '09) showed that problems of this kind can be approached by determining the correlation between the total organs laid down and the deviation of the number of a particular kind from the probable number on the assumption that the proportion of the particular kind is independent of the total number.

The statistical method may of course be applied to experimental data or to series of determinations made on organisms developing under natural conditions. As yet experimental series are not available.

In a former paper ('16) I showed that in the inflorescences of both *Arisarum vulgare* and *A. proboscidium* there is a significant negative correlation between the total number of flowers and the deviation of the number of staminate flowers from their probable number on the theory of proportional distribution. Thus the male flowers while *absolutely* more numerous in the inflorescences with larger total numbers of flowers are *relatively* less numerous than in the inflorescences with smaller total numbers of flowers. Or, conversely, the larger inflorescences tend to produce *a larger proportion* of pistillate flowers.

In this paper the same analytical methods will be applied to the problem of the relationship of the number of stamens and the number of pistils to the total number of stamens and pistils produced by the flower.

II. Materials.

The materials upon which the coefficients discussed in this paper are based have been tabled and the chief biometric constants deduced by competent statisticians. The special methods upon which the conclusions of this paper are based were not, however, available at the time their calculations were made. The results are, therefore, quite new.

The series employed are the following:

1–2. A series of 283 countings of number of stamens and

pistils of *Ficaria verna* from Trogen and another series of 80 countings from Gais, published by Ludwig ('01). Statistical constants for both of these series have been deduced and published by Dr. Alice Lee ('02).

3–4. A series of 268 early and 373 late flowers of *Ficaria ranunculoides* collected by MacLeod ('99) and discussed by W. F. R. Weldon ('01).

5–8. Four series of *Ficaria ranunculoides* collected by Galton, Weldon, Pearson ('03) and others in Italy, Guernsey and England.

III. Presentation of Data.

The means and variabilities of number of stamens and pistils per flower have been given in the papers cited. The only point which requires discussion in this place is the relative variability of the number of the two types of sporophylls. This is shown in Table I.

Table I.

Relative Variabilities in Number of Stamens and Number of Pistils in *Ficaria*.

Series.	Number of Flowers.	Coefficient of Variation for Pistils.	Coefficient of Variation for Stamens.	Differences
Switzerland—				
Trogen, I.	283	23.32	18.68	4.64
Gais, II.	80	23.73	12.18	11.55
Belgium—				
Early, III.	268	23.32	14.07	9.25
Late, IV.	373	27.89	18.46	9.43
Italy, V.	624	22.35	14.12	8.23
Guernsey, VI	520	26.54	17.16	9.38
England—				
Dorset, VII.	505	26.38	16.84	9.54
Surrey, VIII.	500	27.19	17.32	9.87

The number of pistils is consistently more variable than the number of stamens.

Other workers have shown that there is a correlation of medium intensity between the number of stamens and the number of pistils per flower. Their constants, all of which have been rechecked in the course of this work, are shown in Table II. I have also added the linear regression equations showing the rate of increase in mean number of pistils associated with an increase in the number of stamens and the rate of increase in

TABLE II.

CORRELATION BETWEEN NUMBER OF STAMENS AND PISTILS IN *Ficaria* AND REGRESSION EQUATIONS FOR STAMENS AND PISTILS.

Series.	Number of Flowers.	Correlation Stamens and Pistils.	Ratio of Correlation to Probable Error r/E_r.	Regression Line.
Switzerland—				
Trogen	283	.530 ± .029	18.27	$S = 11.708 + .645\,P$
				$P = 4.461 + .429\,S$
Gais	80	.388 ± .064	6.06	$S = 19.075 + .262\,P$
				$P = 4.403 + .575\,S$
Belgium—				
Early	268	.507 ± .031	16.35	$S = 18.197 + .489\,P$
				$P = 3.427 + .525\,S$
Late	373	.749 ± .015	49.93	$S = 9.006 + .729\,P$
				$P = -1.593 + .769\,S$
Italy	624	.439 ± .022	19.95	$S = 19.313 + .418\,P$
				$P = 5.409 + .460\,S$
Guernsey	520	.534 ± .021	25.43	$S = 18.302 + .404\,P$
				$P = 4.160 + .707\,S$
England—				
Dorset	505	.669 ± .017	39.35	$S = 20.369 + .535\,P$
				$P = -1.333 + .835\,S$
Surrey	500	.660 ± .017	38.82	$S = 19.091 + .588\,P$
				$P = -.860 + .741\,S$

mean number of stamens associated with an increase in number of pistils per flower.

The regression of the number of stamens on the number of pistils and of the number of pistils on the number of stamens is shown for three of the larger series in Figs. 1–3.

The empirical means for the Italian series, Diagram 1, do not conform very satisfactorily to the lines given by the equations. Better agreements between actual and theoretical means could hardly be found (in series of data no larger than these) than in the Guernsey and Surrey series represented in Figs. 2 and 3.

The main purpose of the present paper is to present the results of the determination of the relationship between the total number of sporophylls and the number of stamens and pistils.

The correlations between the total number of sporophylls and the number of stamens and pistils are shown in Table III.

As is to be expected the correlations between total sporophylls and number of stamens and pistils are high.

The constants showing the relationship between the total number of sporophylls and the deviation of the number of

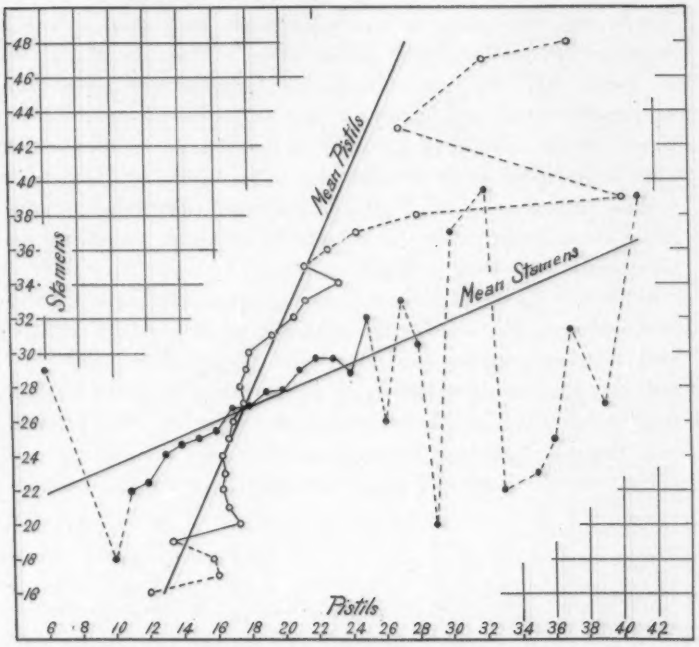


FIG. 1. Average numbers of stamens in flowers with various numbers of pistils and average numbers of pistils in flowers with various numbers of stamens. Empirical and smoothed values. Italian series.

TABLE III.

CORRELATION BETWEEN TOTAL SPOROPHYLLS AND NUMBER OF STAMENS AND PISTILS AND BETWEEN TOTAL SPOROPHYLLS AND DEVIATION OF THE NUMBER OF STAMENS AND PISTILS FROM THEIR PROBABLE VALUE.

Series.	Correlation Between Sporophylls and Stamens.	r_{zz_s}.[1]	r/E_r.	Correlation Between Sporophylls and Pistils.	r_{zz_p}.[2]
I.	.901 ± .008	− .139 ± .039	3.52	.845 ± .012	+.139 ± .039
II.	.755 ± .032	− .548 ± .053	10.37	.896 ± .015	+.548 ± .053
III.	.862 ± .011	− .378 ± .035	10.69	.873 ± .010	+.378 ± .035
IV.	.933 ± .005	− .477 ± .027	17.74	.936 ± .004	+.477 ± .027
V.	.840 ± .008	− .354 ± .024	14.99	.855 ± .007	+.354 ± .024
VI.	.836 ± .009	− .433 ± .024	18.04	.910 ± .005	+.433 ± .024
VII.	.892 ± .027	− .487 ± .023	21.37	.932 ± .004	+.487 ± .023
VIII.	.900 ± .006	− .463 ± .024	19.64	.921 ± .005	+.463 ± .024

[1] Correlation between sporophylls and deviation of stamens from their probable value.

[2] Correlation between sporophylls and deviation of pistils from their probable value.

macro- and the number of microsporophylls from their probable value are the coefficients of critical value. These are also given in Table III. The correlations for stamens and pistils are necessarily equal in magnitude but opposite in sign. They show that the number of pistils is relatively larger in the flowers with larger numbers of sporophylls. The results are consistent in sign throughout. All of the correlations except that for the series from Trogen may be considered certainly significant in comparison with their probable errors.

While the constants for certain of the series differ significantly, the results are (considering the relatively small numbers and the very wide geographical distribution of the material) very consistent. Five of the eight series differ from $r = \pm .50$ by less than twice their probable error. Of the other three series, only Professor Ludwig's Trogen material is very aberrant.

For two of the series I have determined the standard devia-

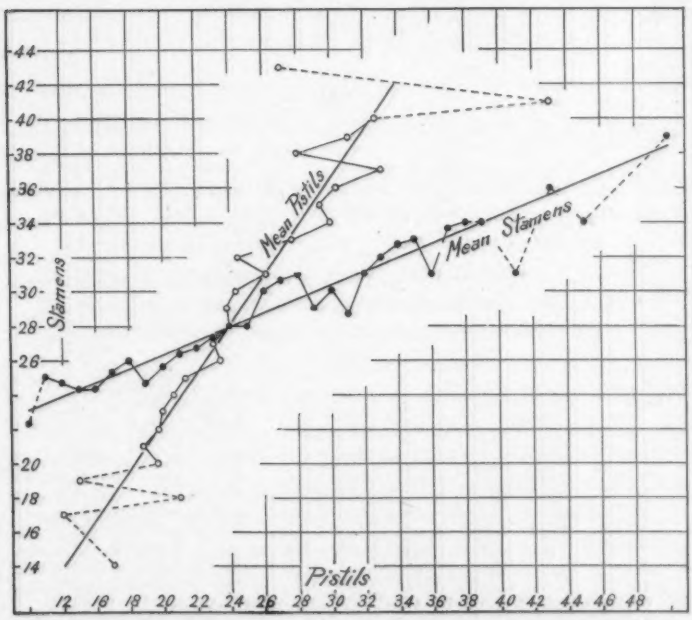


FIG. 2. Empirical means and regression straight lines for regression of stamens on pistils and pistils on stamens. Guernsey series.

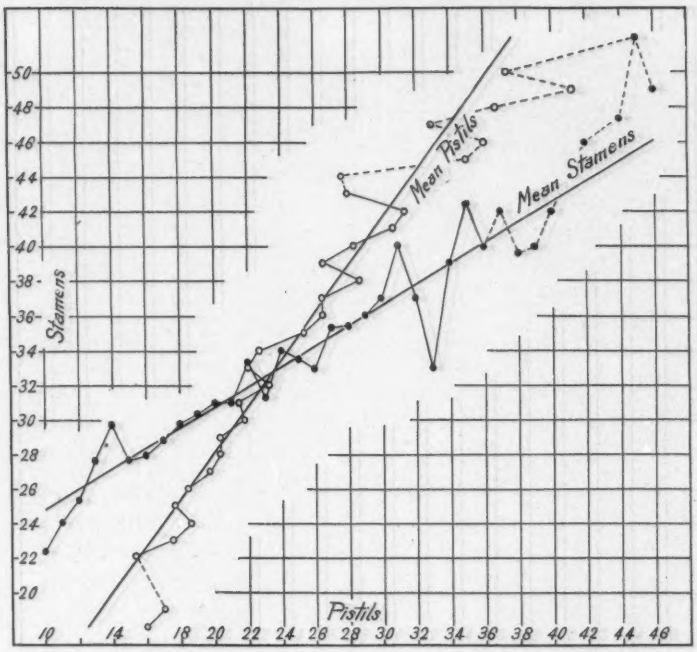


FIG. 3. Explanation as in Figs. 1 and 2. English series.

tion of the deviation of the number of stamens (or pistils) from their probable value by a formula to be published shortly ('17). They are:

For Bordighera, $\sigma_z = 2.1873$
For Surrey, $\sigma_z = 2.8054$

These values make possible the determination of the straight line equations showing the regression of the deviation of the number of stamens and pistils from their probable values upon the total number of sporophylls. They are:

For Bordighera series—$Z_s = +5.2446 - .1181\,S$
$Z_p = -5.2446 + .1181\,S$
For Surrey series— $Z_s = +6.6565 - .1179\,S$
$Z_p = -6.6565 + .1179\,S$

Here Z_s and Z_p denote the deviations of the stamens and pistils from their probable values and S denotes the total number of sporophylls.

The results are represented graphically in Figs. 4 and 5. In the Italian series, flowers with fewer than 36 stamens and pistils are only 29 in number, distributed among flowers with from 28–35 sporophylls per flower. The means at this end of the range cannot, therefore, be expected to show great regularity.

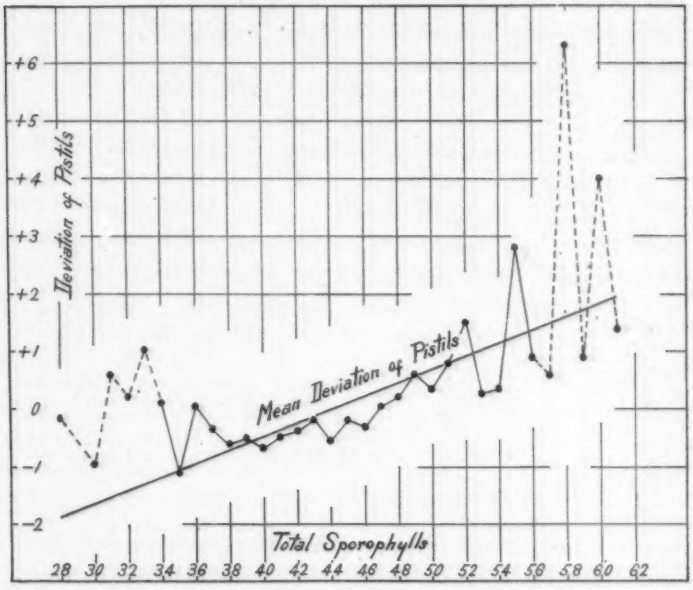


FIG. 4. Regression of the deviation of the total number of pistils from their probable value on the total number of sporophylls. Italian series.

Flowers with more than 61 sporophylls are only 10 in number but are distributed among flowers ranging from 62–85 sporophylls per flower. This portion of the range has not been included in Fig. 4. The relationship is apparently not quite linear.

In the material from Surrey, shown in Fig. 5, the increase in the relative proportion of pistils associated with increase in the total number of stamens and pistils could hardly be better represented than by the slope of the straight line indicated by the equation.

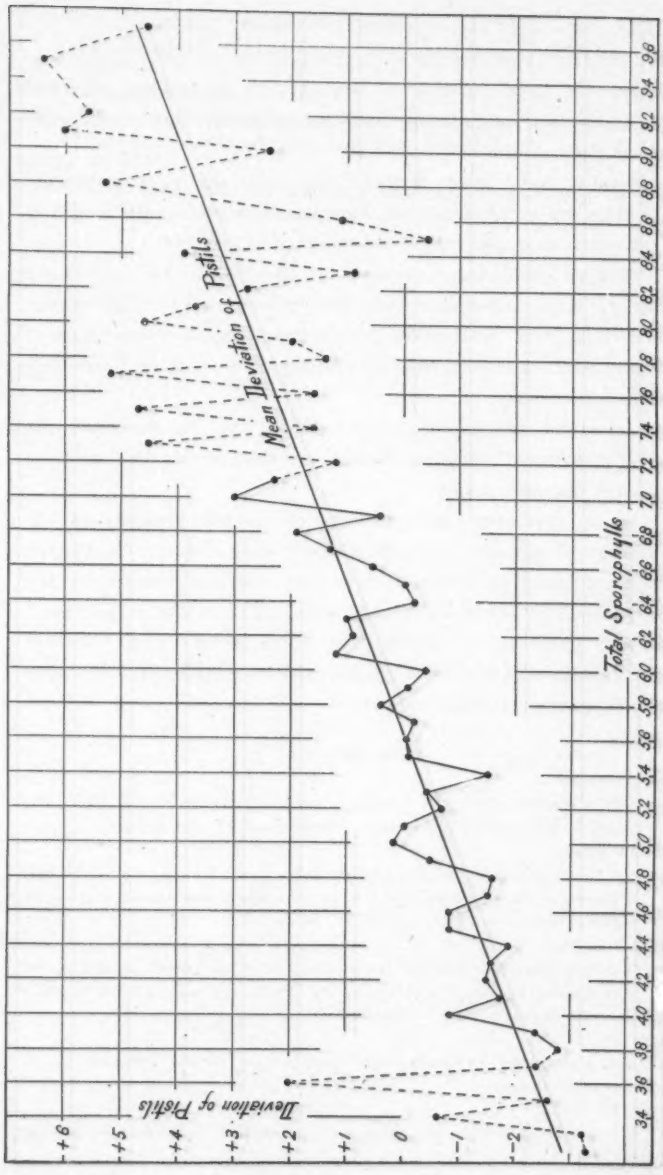


FIG. 5. Regression of the deviation of the total number of pistils from their probable value on the total number of sporophylls. Surrey material.

IV. Recapitulation.

The present study deals with the problem of the relationship between the total number of sporophylls laid down and the relative number of stamens and pistils in the ranunculaceous genus *Ficaria*.

Constants have been deduced for eight series of published data from Italy, Switzerland, Belgium, Guernsey and England not hitherto analyzed by the methods now available.

In flowers with larger numbers of sporophylls the pistils are relatively more numerous than the stamens. The high degree of consistency of the results drawn from such a wide range of habitats indicates that the relationship is one of real morphogenetic significance.

In an earlier paper it has been shown that in *Arisarum* the relative number of pistillate flowers increases as the total number of flowers becomes larger.

It is at least suggestive in relation to the problem of the physiology of sex that in both of these very different forms the number of macrosporophylls becomes relatively larger as the total number of sporophylls increases. The relationships may, however, have an embryological explanation. Only further investigations will justify final conclusions concerning the cause of the relationship demonstrated.

References.

Harris, J. Arthur.

'09 The Correlation between a Variable and the Deviation of a Dependent Variable from its Probable Value. Biometrika, VI., pp. 438–443.

Harris, J. Arthur.

'16 On the Distribution and Correlation of the Sexes (Staminate and Pistillate Flowers) in the Inflorescence of Arisarum vulgare and Arisarum proboscidium. Bull. Torr. Bot. Club, XLII., pp. 663–673.

Harris, J. Arthur.

'17 Further Illustrations of the Applicability of a Coefficient Measuring the Correlation between a Variable and the Deviation of a Dependent Variable from its Probable Value. Shortly.

Lee, A.

'02 Dr. Ludwig on Variation and Correlation in *Ficaria*. Biometrika, I., pp. 316–319, 1902.

Ludwig, F.

'01 Variationsstatistische Probleme und Materialen. Biometrika, I., pp. 11–29.

MacLeod, J.

'99 Over de correlatie tusschen het aantal meeldraden en het aantal stampers bij het Speenkruid (Ficaria ranunculoides). Botanisch. Jaarboek, XI., 1899.

Pearson, K., and others.

'03 Coöperative Investigations on Plants, II. Variation and Correlation in Lesser Celandine from Divers Localities. Biometrika, II., pp. 145–164.

Weldon, W. F. R.

'01 Change in Organic Correlation of Ficaria ranunculoides During the Flowering Season. Biometrika, I., pp. 125–128.

SOME OBSERVATIONS ON ARTEMIA GRACILIS, THE BRINE SHRIMP OF GREAT SALT LAKE.[1]

ALBERT C. JENSEN.

Very little investigation has been made of the animal life of Great Salt Lake. This is probably because in popular literature we still read that no living thing can exist in the brine of the lake, or because the few forms adapted to such life have failed to interest scientists. So far as the author knows, no attempt has been made to make a complete scientific classification of the lake fauna or to do any extensive research work on it. The brine shrimp, *Artemia gracilis*, one of the lake forms, is a small crustacean found in great abundance.

In referring to the literature on the subject, we find that Captain Bonneville wrote short descriptions of his explorations along the shores of Great Salt Lake, making mention of small animals in the water, probably the brine shrimp, as early as 1831–1833. Fremont also states of rowing to an island in the lake where he found what he called larvæ of insects washed upon the shore in great heaps or windrows three or four feet wide, and many animals living in the water. Verrill described the brine shrimp as *Artemia gracilis* in 1869. Seven years later Dr. Siebold, of Munich, obtained through Dr. Hagen, of Cambridge, Mass., a quantity of dried mud from Great Salt Lake which contained fertile eggs of *Artemia*. These eggs were hatched in artificially prepared brine and shrimps reared therefrom. Siebold succeeded in propagating *Artemia* for several generations and was quite convinced that this crustacean reproduces parthenogenetically as well as sexually. Packard ascribes the abundance of this phyllopod in Great Salt Lake to the absence of enemies. Further, after an examination of specimens he concluded that Verrill's later described species are merely different stages of the same species and should be included under the first described form, *Artemia gracilis*. As late as 1889 David Starr Jordan stated

[1] Contribution from the Zoölogical Laboratory of the University of Utah.

that the lake water was so salty that no life could exist in it except the brine shrimp. Gilbert suggests that the presence of *Artemia* is no mystery for its ancestors may have lived in the fresh water of the basin and have transformed with the increasing saltiness of the lake. Talmage observed that the little phyllopod existed in great numbers in the lake, especially between the months of May and October. He states having collected them in the midst of winter when the temperature was far below the freezing point of fresh water, and from the evaporating ponds of the salt works, where the brine was near the point of saturation. He was able to keep them alive for several days in various dilutions of lakewater, and noted that they would even live for some time in distilled water. He further observed that repeated washings in fresh water for five minutes removed the brine so completely that salt had to be added to make them a palatable food.

The adult *Artemia*, Plate I., has a small head carrying a single black median eye (ocellus), a pair of large stalked compound eyes, two pairs of antennæ, one pair of mandibles and a pair of maxillæ. The second pair of antennæ of the female are short and pointed, while those of the male are large and broad, Plate II. The thorax bears eleven pairs of legs which are leaf-like in structure and serve as swimming organs and for the attachment of the respiratory organs. The abdomen is long, composed of eight segments without appendages. A number of bristles are to be found on the terminal segment. The ovisac is roughly bottle-shaped, being rather short and broad and attached to the first and second abdominal segments.

The œsophagus is short and leads to the stomach, which is situated in the head. The stomach is divided into two lobes, each of which receives a duct from the liver. The liver is two-lobed and lies in the head anterior to the stomach. The intestine is a straight tube. The division extending through the thorax is twice as large as that passing through the abdomen. The intestine includes a well-marked rectum, which is provided with constricting circular muscles. The ovaries are located in the first and second abdominal segments ventral to the intestine. The testes are located in the same relative position. The heart

is a long tube beginning just posterior to the mandibles and terminating in the anterior part of the last abdominal segment. It has a distinct valve at the posterior end and apparently a pair of valves for each segment, Fig. 1. The blood enters the heart through these side ostea and is forced out through the ends of the heart. The central nervous system consists of a two-lobed supraœsophageal ganglion in front of the œsophagus, and just behind the œsophagus, between the digestive canal and the floor of the body, is the subœsophageal ganglion, the first of a chain that extends through the body along the ventral side. Nerve fibers extend from these ganglia to the body and appendages.

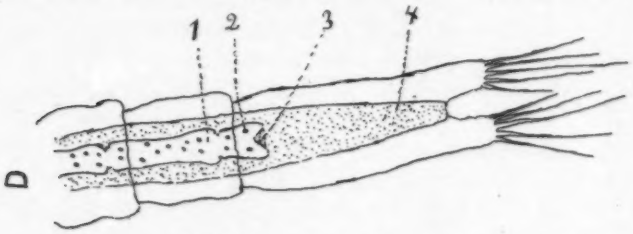


FIG. 1. Last three abdominal segments. 1, Heart; 2, blood corpuscles; 3, terminal valve; 4, intestine.

The sexes are separate in *Artemia*. The female is 10 to 12 mm. long, while the male has a length of 8 to 10 mm. Reproduction is accomplished by means of eggs that may or may not be fertilized.

In copulating the male claspers are placed around the body of the female just in front of the ovisac and thus attached the pair may swim about for several days, as many as five having been observed. At intervals the male's abdomen may be seen to bend so that the genital appendages come into contact with the oviduct. Frequently copulation takes place before the ovisac begins to show a brown color, due to the color of the eggs, and in such cases the eggs are retained in the ovisac for as long as three days after copulation has ceased. In other instances copulation does not take place until the eggs are brown in the ovisac and often these are deposited and even hatched while copulation is still in progress. It is presumed that such a copulation is too

late to fertilize the eggs for they have passed from the ovaries into the ovisac and are covered with a brown shell and are ready to be deposited. These eggs, deposited during April and May, hatch within twenty-four hours after being laid.

A comparison of these newly deposited eggs with those collected during the early spring showed no particular difference, except that none of the new eggs were cup-shaped as are many of the winter eggs, Plate III., *A*. Examination of a number of females showed the number of eggs to vary between sixteen and one hundred and twenty. The females deposit their eggs while swimming.

Eggs were collected March 8 and kept in the laboratory in normal lake water for twenty-one days. The room temperature apparently had little effect upon the development since nauplii were collected in the lake three days after individuals were observed in the aquarium.

The embryo transforms into a nauplius much unlike the adult, Fig. *E*, Plate III. It has a blood-red color, a median eye (ocellus) and three pairs of appendages, the first of which develop into the first pair of antennæ, the second pair are much larger and serve as swimming organs, while the third pair become the mandibles. After a few days the second pair, in females, become shorter, less movable, lose their bristled margins, and are transformed into small scarcely movable processes, the second antennæ, Plate II., *B*. In the males they also form the second antennæ which develop into disproportionately large claspers with broad lobes, Plate II., *A*, and are used as catching and clasping organs. Along with the early development of these are signs of segmentation which is followed by the appearance of the thoracic appendages one pair after another until all eleven pairs have been laid down. The stalked compound eyes appear about the same time as the first thoracic appendages. Within eighteen to twenty-one days the animals reach sexual maturity, shown by copulation. Sexual differences become apparent when the animals are a little more than half grown. The males are stronger and more active than the females.

Siebold's conclusion that *Artemia* may reproduce parthenogenetically is verified by my observations. Water was taken

from the lake before nauplii appeared there, and precautions taken to eliminate all eggs. Undeveloped females were placed in this water, where they developed normally and deposited eggs similar in appearance to fertilized ones. These eggs produced males and females, which developed normally and as far as could be observed could not be distinguished from those coming from fertilized eggs. A large proportion of the hatch was females. Both methods are evidently used during the summer months.

According to my records all of the brine shrimps die during the winter months, probably due to the lowered temperature. These observations do not harmonize with those of Talmage, who says: "I have taken them in the midst of winter, when the temperature of the water was far below the freezing point."

Statements made by Gilbert that the ancestors of *Artemia* may have lived in the fresh water of the basin, led to an experiment to determine the effect of different concentrations of salt water upon this animal. By using distilled water and the process of evaporation, solutions were prepared having a specific gravity as follows:

I.	II.	III.	IV.	V.	VI.	VII.	VIII	IX.
Dis. water					Normal lake water	Evapo-rated to	Evapo-rated to	Evap. to saturated point
1.000	1.027	1.044	1.064	1.085	1.109	1.130	1.711	1.179[1]

On March 28 eggs were put into the above solutions. March 30, 44 hours later, nauplii had emerged and were swimming about in solutions 1, 2 and 3 and in 4 they were emerging from the eggs. In the latter I observed the ocellus, also one or two pairs of appendages that were moving as if trying to free the body from the shell. By April 10 nauplii were emerging in solution 5 and not until April 13 did the eggs in solution 6 begin to hatch. By this date nauplii were also emerging from eggs in the lake. On May 5 a few nauplii appeared in solution 7, but in solutions 8 and 9 no nauplii had emerged. To test if the eggs in these last two solutions were fertile, a few were put into a less dense solution where they hatched readily. In solution 1

[1] The specific gravities herein given are the uncorrected hydrometer readings.

eggs hatched readily in twenty-four hours, but the nauplii lived only from one to three days. However transferring nauplii from 1 to solutions 2 to 6 inclusive brought good results. In solutions 2 to 6 the nauplii, hatched in the order stated above, lived and developed rapidly to maturity. Young and adult *Artemia* transferred to solutions 8 and 9 from less dense solutions died within five to ten hours, the young dying first. Those put into solution 7 developed slowly, while those placed into solutions 2 to 6 developed quite normally. Solutions 3, 4 and 5 however seemed to be the most favorable for the growth of *Artemia*. They would also develop normally when transferred among any of the solutions 2 to 6 inclusive.

Artemia is often found in great numbers. Fremont in writing of his explorations of the lake speaks of windrows of the brine shrimp and the pupa cases of insects washed upon shore. Talmage states having seen the surface of the water tinted by them and also of having gathered with a tow-net a quart within a few minutes. Indians inhabiting this region used to collect large quantities of the crustacean along with insect larvæ which they dried and used as food.

The brine shrimp is a vegetable feeder. Near shore may be seen many masses of an alga, *Aphanothece*, and it is along with this that *Artemia* is most abundant. This alga and its gelatinous secretion forms the principal food for the phyllopods as shown by the fact that they may be seen feeding upon it, and it can also be demonstrated in their digestive tracts. In fact this alga seems to be their only food.

Artemia gracilis is chiefly a littoral form, since in this zone its food is most abundant. However these crustaceans have been observed by Talmage in great swarms far out in the lake where they were carried by winds unless they have swarming or migratory habits.

There is a very disagreeable odor encountered upon approaching the shores of Great Salt Lake at certain places. This stench has been attributed to the decomposition of the brine shrimp. The odor is at times very offensive, which, by some, is thought to be dangerous, and as a result the summer resorts of the lake are not visited by many people who would otherwise enjoy them.

The stench is due primarily to decaying vegetable matter. Vessels placed in the laboratory containing plant material from the lake gave off the characteristic lake odor. Further the odor was present at the lake as early as the last of March, before the appearance of *Artemia*. Again the proportion of the phyllopods to the plant material is so small as to make the brine shrimp an insignificant factor in the odor production.

Conclusions.

Winter eggs of *Artemia* kept in normal lake water at room temperature (about 20 degrees centigrade) for a period of two months did not hatch earlier than those in the lake.

The embryo of *Artemia* develops into a free-swimming nauplius, much unlike the adult, which passes through a series of stages before reaching maturity.

Artemia reproduces by means of fertilized eggs and also parthenogenetically.

Eggs will hatch during the winter as well as spring and summer in dilute lake water or in fresh water at a temperature as low as 20 degrees centigrade. My experiments show also that eggs will not hatch in solutions concentrated to near the saturation point.

Artemia passes the winter months in the egg stage.

Artemia will not live in fresh water, but will in a dilution of the lake water having a specific gravity of 1.027.

Lake water diluted to a specific gravity of 1.044 to 1.027 is most favorable for the development of *Artemia*.

I take this opportunity to heartily thank Dr. Newton Miller for his assistance in this work.

REFERENCES

Fremont, John C.

'43 Report of the Exploring Expedition to the Rocky Mountains in the Year 1842, and to Oregon and North California in the Years 1843–4. Washington, D. C., 1845.

Verrill, A. E.

'69 Twelfth Annual Report U. S. Geological and Geographical Survey of the Territories of Wyoming and Idaho, 1878, Part 1, page 330. Washington, D C.

Siebold.

'78 Twelfth Annual Report U. S. Geological and Geographical Survey of the Territories of Wyoming and Idaho, 1878. Part 1, pages 459–463. Washington, D. C.

Packard, A. S., Jr.

'78 Twelfth Annual Report U. S. Geological and Geographical Survey of the Territories of Wyoming and Idaho, 1878. Part 1, pages 295–592. Washington, D. C.

Jordan, David Starr.

'89 Bulletin of the U. S. Fish Commission, Vol. IX., 1889. Report of Exploration of Colorado and Utah during the Summer of 1889, page 31. Washington, D. C.

Gilbert, Grove Carl.

'90 Lake Bonneville, page 259. Washington, D. C.

Talmage, James E.

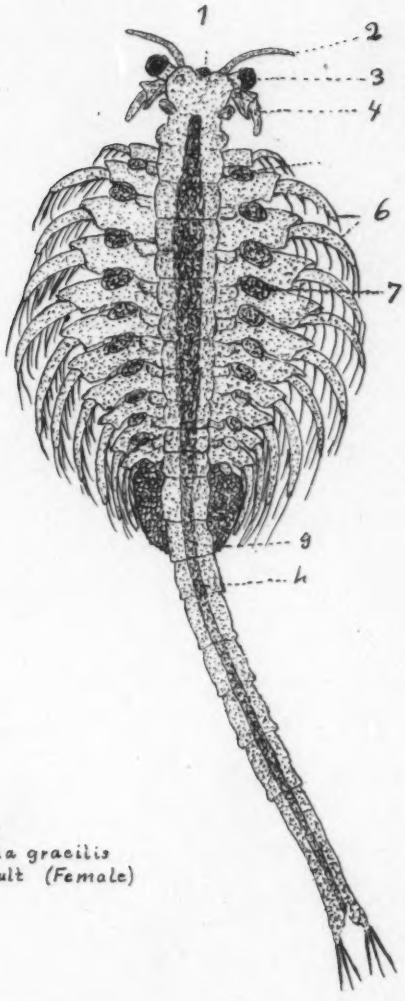
'00 The Great Salt Lake—Present and Past, pages 67–76. Salt Lake City, Utah.

EXPLANATION OF PLATES.

All figures drawn with the camera lucida.

PLATE I.

Adult female *Artemia gracilis*.



Artemia gracilis
Adult (Female)

PLATE II.

A. Head of male showing first pair of antennæ.
B. Head of female showing first pair of antennæ.
C. Mandible.
D. Compound eye.
Figures of this plate drawn on the same scale.

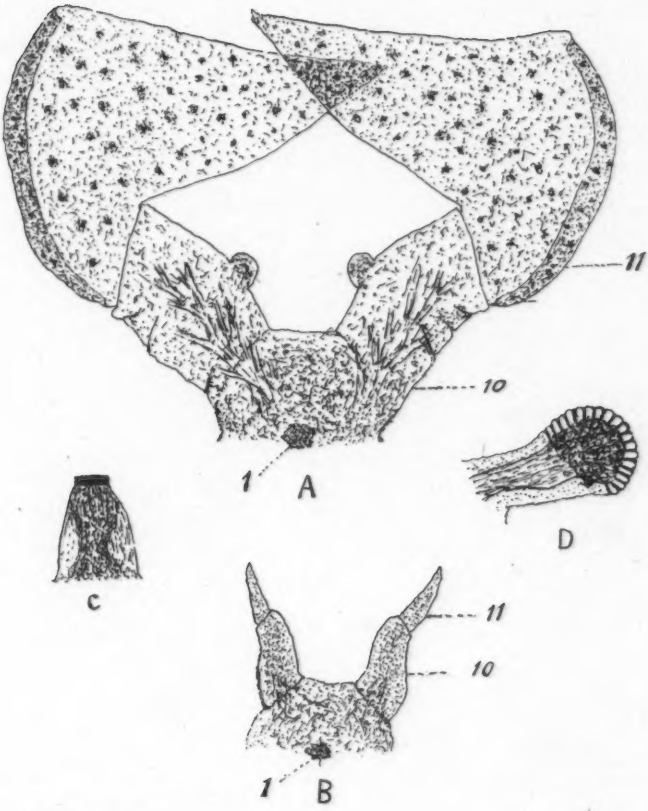


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PLATE III.

A. Cup-shaped egg.
B. A spherical egg.
C and *D*. Nauplii emerging from eggs.
E. Early nauplius stage.

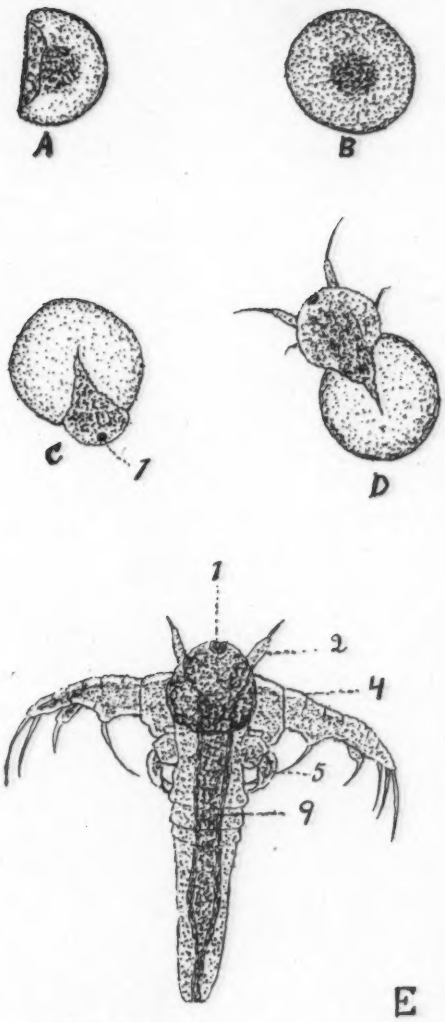

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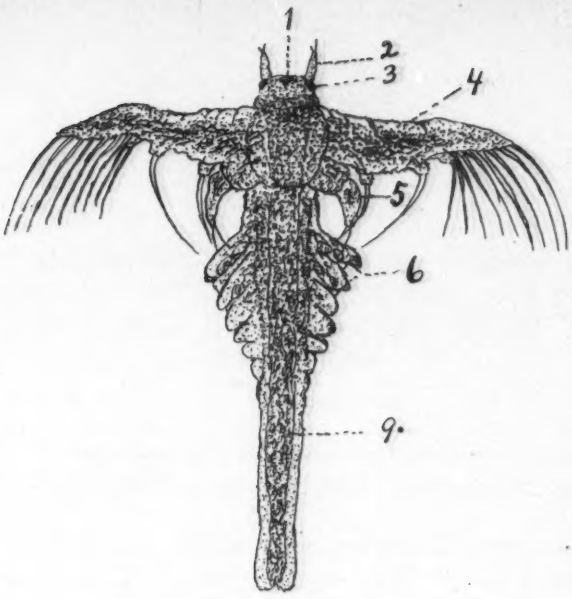
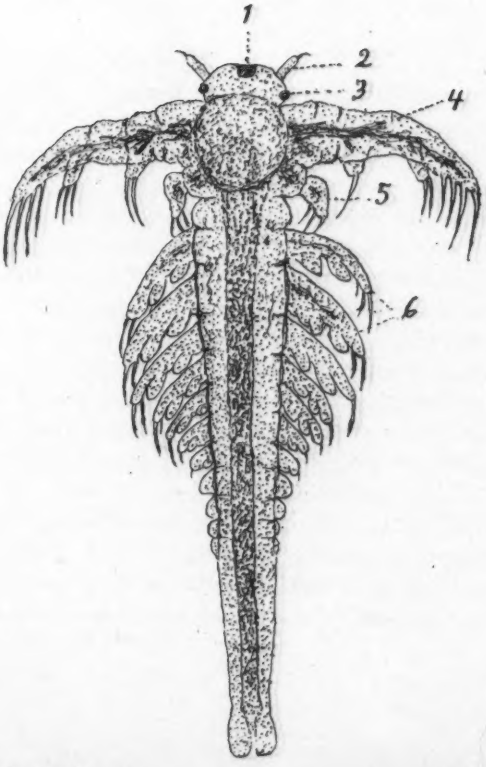
PLATE IV.

Later nauplius stages.
Figures of plates III. and IV. drawn on the same scale.
Notations.

1. Ocellus.
2. First antenna.
3. Compound eye.
4. Second antenna.
5. Mandible.
6. Thoracic appendages.
7. Gills.
8. Ovisac.
9. Intestine.
10. Basal segment of the first antenna.
11. Distal segment of the first antenna.





ALBERT C. JENSEN.

THE RELATIONS BETWEEN THE INTERSTITIAL GLAND OF THE TESTICLE, SEMINIFEROUS TUBULES AND THE SECONDARY SEXUAL CHARACTERS.[1]

LEO LOEB.

The following observations may prove of interest in the analysis of the relations between male gonads and secondary sexual characters.

While, as is often the case, an experiment of nature, unaided by experiments in the laboratory, is unable to give a definite answer to the questions which it raises, yet it would at present be impossible to duplicate at will in the laboratory an experiment such as we observed and it is, therefore, desirable that it should be recorded.

Our observations were as follows:

Guinea pig No. 1523 had been sent to our laboratory by an animal breeder as a female that had apparently been in heat, but in some respects behaved abnormally. The report of the breeder was as follows:

"The female through her actions gave every sign of being in heat; but upon examining her, she did not look to me as if she really was in heat. I had her with two other females and she would mount them just the same as a male guinea pig would do when in the act of copulation and she would also allow the other females to act in the same way. She would stand in this position, meanwhile expanding the vagina, which generally is a sure sign of their being in heat. I would say that she is with young; at least she should be pregnant, because she had been with a male for some time. Expect some young at any time."

The breeder made his observations July 8, 1917. On July 12, 1917, the animal, which weighed 615 grams, was prepared for operation. Under ether anesthesia lumbar incisions were made,

[1] From the Department of Comparative Pathology, Washington University Medical School, St. Louis, Mo.

in order to expose the ovaries. Typical ovaries were, however, not found. Instead, we encountered near the place where the ovaries usually are situated, but perhaps a little farther down, somewhat large and round, rather soft bodies, each about the size of a pea, surrounded by much fat tissue. On cutting through the center the color was found to be slightly yellowish-brown. From here some fibrous bands extended downward in the direction in which the uterine horns are usually situated. An organ that resembled the uterus was not found. The vagina was also lacking. Neither was a penis, vas deferens or a descended testicle visible.

The brownish red bodies as well as pieces of the fibrous bands were fixed in Zenker. Both of the bodies were completely cut into serial sections. Tissue from the places where the mammary glands are usually situated was also fixed and sectioned.

Microscopic examination:

1. The round bodies situated below the kidney were seen, microscopically, to consist of testicle tissue in which there was an extraordinarily marked development of interstitial gland. The testicle tubules were lined by one layer of epithelial cells, the outline of which was not very definite. Towards the center of the tubules they formed a network of fine fibrils; a sharply defined lumen therefore did not exist in the tubules. The cells had either a cuboidal or cylindrical form as far as their indefinite outline permitted of such a characterization. In some cases the whole protoplasm of the cell was drawn towards the center of the tubule in a tail-like structure. The nucleus was situated in the center of the cell and was very characteristic. It represented a clear vesicle in which one deep-staining large nucleolus was visible. Occasionally the nucleolus divided into two parts. In various places the tubules were lined with several cell rows. Sometimes the cytoplasm and the nucleus of one cell swelled, and such a cell encroached upon the territory of the neighboring cell, lying more towards the center of the tubule, pressing against it; and thus one cell surrounded, crescent like, the neighboring cell. Occasionally some cells degenerated, the nucleolus persisting longest, while the rest of the nucleus had already disappeared. Mitoses were seen quite frequently in these tubule cells and monasters as well as diasters

were found. The tubules were enveloped by a circular layer of flat connective tissue cells, which were drawn out into long fibers with flat nuclei. Each tubule was surrounded by several, usually two to five, layers of interstitial cells. Each tubule with the surrounding concentrically arranged interstitial cells formed a group which was often quite sharply separated from neighboring similar groups through lymph vessels or lymph-spaces and blood vessels and accompanying connective tissue strands. In certain places larger blood vessels accompanied by lymph vessels were found in the tissue. Blood capillaries were numerous in certain places. Occasionally a tubule was found adjoining the connective tissue directly, without being separated from the latter by interstitial cells. The character of the interstitial cells varied somewhat in different parts of the organ. Usually they were large polygonal, partly rounded off or oval cells with a large vesicular nucleus, in which several small nucleoli were generally seen, instead of the one clear nucleus which was so characteristic of the tubule cells. The nucleus in the interstitial cell was not so light and transparent as in the tubule cell. The sizes of the interstitial cells varied. The cytoplasm became finely vacuolar, at first in the periphery. In the center there was still a solid material staining red with eosin. Later the whole cytoplasm became finely vacuolar. This vacuolization was usually accompanied by a considerable increase in size of the cells. In some cases the vacuoles enlarged still more, the walls separating neighboring vacuoles disappeared and irregular cavities became visible in the cells. In those cases the nucleus became irregular and shrank, and when this last stage had been reached, the cell was evidently degenerating. Many finely vacuolar enlarged cells appeared otherwise normal and viable. These finely vacuolar, swollen cells occurred especially around the lymph vessels, or perhaps also around certain blood vessels. We must assume that substances reaching the cells from the circulation were deposited within the cells and caused the vacuolization, the nearness to the source of this substance determining the frequency and intensity with which these changes took place. Under the same conditions the accompanying fibrous tissue became edematous. Apparently the vacuolization of the interstitial cells and the edema-

tous state of the stroma are analogous phenomena. We were only rarely able to find mitoses in the interstitial cells. In this respect the latter differed from the tubule cells in which mitoses were quite frequent. There could be found at one end of each testicle a system of branching epithelial ducts, with a narrow lumen. They were lined by a layer of small and densely packed, usually cuboidal or flat epithelial cells, with nuclei which filled the greater part of the cells. In these cells either one or several nucleoli were found. If one nucleolus was present the nucleus resembled that of the tubule cells, except that it was smaller. At other places the lining cells and nucleus were low cylindrical. In the lumen of these ducts not infrequently some invaginations of the epithelium were found. On the one side the ducts formed a union with the tubule of the testicle. Between the two we found transitional structures consisting in part of tubular and in part of duct epithelium. At the other end the ducts entered the fat tissue which surrounded the testicle and made connection with some larger ducts. One change that took place in a number of tubules needs special mention: A few or many, at some places even the majority of the tubule cells underwent certain changes which made them very similar to interstitial cells. They enlarged, became polygonal or somewhat round, and their cytoplasm stained more strongly with eosin. The cytoplasm became finely vacuolar, at first the periphery and later the greater part of the cell undergoing this change. At the same time the nucleoli divided into several particles which were dispersed in the nucleus. The nucleus became a round vesicle which had lost the transparency so characteristic of the nucleus of the tubule cells. Accompanying this change the layer of flat connective tissue cells which separated tubules and interstitial cells disappeared and at various places interstitial cells adjoined directly the tubule cells, both kinds having become very similar. Such changes seemed to be frequent especially in places where the interstitial cells were swollen and had become finely vacuolar. Three interpretations of this condition suggest themselves:

1. As a result of their enlargement the interstitial cells push into the tubules, and thus the appearance within the tubules of typical tubule cells adjoining directly cells resembling interstitial

cells might be explained. At the same time the enlarged interstitial cells exerted a pressure on the tubule cells which made them crowd closer together than usual. This interpretation is not admissible, because the cells within the tubule began to swell at a time when the connective tissue layer separating tubule and interstitial tissue was not yet broken through. At first the enlarging cells still possessed the nucleus characteristic of the tubule cells and only gradually the character of the nucleus changed concomitantly with the later stages of the transformation.

2. It might be assumed that an actual transformation of tubule tissue into interstitial tissue took place, and that the gland-like interstitial tissue was merely further differentiated tubular gland tissue. Many pictures seemed to suggest such an interpretation. However, the fact that in many other places, especially where the interstitial cells were still solid and smaller, and, therefore, younger, the line of demarkation between tubule and interstitial tissue throughout was very sharp made, after all, this interpretation improbable. Furthermore, it would be contradicted by what is known of the origin of the interstitial cells.

3. It is most probable that the same factors which produce the changes in the interstitial cells, at the same time called forth similar changes in the tubule cells. Probably certain substances furnished by the lymph or blood vessels were simultaneously responsible for both sets of changes. In favor of this interpretation might be cited the fact that we did not usually find in the tubules that extreme vacuolization, which was present in some of the interstitial cells. We are, therefore, inclined to adopt the third interpretation.

The round bodies which we found near the place where normally the ovaries are situated therefore represent undescended testicles. There is a system of ducts leading from the testicle tubules to the surrounding fat tissue. The tubules are relatively simple structures in which spermatogenesis does not take place. The epithelium lining them corresponds to the sustentacular cells of the normal testis. There is present an extremely marked development of the interstitial cells. The structure of this testicle differs, therefore, markedly from the normal testicle of the

guinea pig which consists principally of tubules with several layers of epithelium which are in the process of spermatogenesis. Between these tubules there is very little stroma with blood vessels and a very small number of polygonal cells.

Sections through the tissue, taken from the place where normally the uterus is situated, show fibrillar connective tissue in which are situated nests composed of rather large polygonal, cylindrical or ellipitical cells, the protoplasm of which stains well with eosin. The nuclei of these cells are round vesicle with chromatic particles distributed rather diffusely. These cells form the matrix of the neighboring fibrillar connective tissue. Through rarefaction of the cytoplasm and through a condensation, backing together of the nuclear chromatin these cells are transformed into the surrounding connective tissue. We also studied in microscopic sections the fat tissue which filled the space between the undescended testicle and the kidney, without finding any trace of further testicular or of ovarian tissue.

Mammary Gland.—The nipples of the guinea pig were distinct and we cut out pieces from the tissue where normally the mammary gland is situated. In the male a few scattered ducts lined by epithelium in which mitoses do not usually occur represent the mammary gland.[1] In our case, a very much furthergoing development of the mammary gland was found on microscopical examination. Sections through one of the two mammary glands showed solid tissue consisting of a large number of lobules of mammary gland tissue, joined together by thin strands of fibrous tissue. The whole gland was surrounded by fat. Each lobule consisted of acini lined by cuboidal epithelium of medium size. The nuclei were vesicular and relatively large. The lumen of the acini varied in size and often contained some colloid material or desquamated cells. Occasionally an acinus cell was found to be vacuolar. In the stroma between the acini there were numerous small connective tissue cells. At some places, where the interstices between the acini were somewhat wider and the stroma was slightly edematous, polynuclear leucocytes staining deeply with eosin were found. The largest ducts were

[1] In the ducts of the mammary gland of a young male guinea pig, about 30 days old, we encountered however one mitosis.

as usual surrounded by denser fibrous tissue. At one pole of the gland a system of ducts and acini lined by higher and larger epithelium extended into the surrounding fat tissue. The lumen of these ducts and acini was wider. Mitoses could not be seen in the mammary gland. The mammary gland of the other side, which was also surrounded by fat, was smaller, at least the amount of gland tissue found on sections, which had been through different parts of the piece, was less; but it still surpassed considerably that found in normal animals. Furthermore, the acini were composed of high cylindrical cells, surrounding a rather large lumen. There were a few desquamated cells in the gland lumina, the acini being arranged around the larger ducts. Here and there mitoses were seen in the acini. The stroma between the acini was loose in texture and contained a number of fibroblasts.

Discussion.—The following are the chief points of interest in these observations:

1. We have in this case apparently to deal with an undescended testicle in an adult guinea pig. Such testicles have been observed in man and several species of animal, especially in the horse and pig. This case is an example of the same condition in the rodentia. Common to all cases which have been so far described is an imperfect development of the seminiferous tubules and a hypertrophy or a hyperplasia of the interstitial cells of the testicle. As to the literature concerning such cases we may refer especially to Bouin and Ancel (1) Tandler and Grosz (2), and Whitehead (3). Both of the features we mentioned are present in a marked degree in our case. Spermatogonia are completely absent; the tubules consist entirely of Sertoli cells. The retention of the testicle acted in a way comparable to the ligation of the vas deferens which brings about similar results which are, however, usually not quite so far going. In addition, it is very probable that the condition which prevented the normal descent of the testicle was directly responsible for the lack of development of spermatogonia. As to the overdevelopment of the interstitial gland, in those cases, we may conclude that it stands in some causal relation and is subsequent to the underdevelopment of the seminiferous tubules. In various other conditions in which atrophy

or underdevelopment of the tubules has been observed, or produced experimentally (ligation of vas deferens, Roentgen ray injury of the testicle (4) (5) and certain diseases) a hypertrophy or a hyperplastic condition of the interstitial gland has been noted. Herxheimer and Hoffman (5) suggest that this hyperplasia is comparable to the substitutive growth of connective tissue which takes place whenever parenchymatous elements are destroyed. This explanation, however, does not seem to take into account the principal variations which have been observed in the condition of the interstitial gland. Bouin and Ancel (1) have shown that in the horse the interstitial gland is present in the fetus; it degenerates in the immature animal and begins anew to develop at the time of puberty. In the sexually mature animal there seems to exist a certain antagonism between the activity of the seminiferous tubules and the interstitial gland. The interstitial gland becomes hyperplastic in cases in which the tubules decrease in activity and vice versa. Under the conditions of tubular atrophy or degeneration which we named above the interstitial gland hypertrophies, and according to Tandler and Grosz in the mole, in which sexual activity follows a yearly cycle, the interstitial gland shows the greatest development at a time when the seminiferous tubules are least active. In hibernating animals the interstitial gland decreases at the period of general metabolic inactivity (von Hansemann); similarly in cases of very pronounced general undernourishment, or of very marked degenerative processes in the testicle the interstitial gland suffers and may disappear. Some observations indicate, furthermore, that under certain conditions extirpation of one testicle may lead to a hypertrophy of the interstitial gland in the second one. All these facts can evidently not be explained otherwise than by assuming that there are several factors active and that chemical and not merely mechanical substitutive factors play a rôle in the regulation of the growth of the interstitial gland. It seems to be a general function of gland and other epithelial cells to stimulate through their activity the surrounding stroma. We have described such an occurrence especially in connection with the cyclic growth of the mammary gland, but it seems to be a phenomenon of general significance. In a similar way we may as-

sume that growth processes in the seminiferous tubules stimulate the activity of the connective tissue cells and those specialized connective tissue cells which have the potentiality of becoming transformed into interstitial gland. This would explain the development of the interstitial gland during embryonic development and at the time of puberty. We might designate this as the concomitant growth of the interstitial gland. In order to explain the second kind of growth in the interstitial gland, which we might call the "alternative growth," we must assume that in a certain way the activity of the sexually active seminiferous tubules exerts an inhibiting effect on the growth of the interstitial gland and that a diminution of this inhibiting effect leads to the "alternative" growth of the interstitial gland. An analogous condition is found in the rabbit ovary in which the atresia of certain follicles leads to a secondary gland-like development of the theca interna. How far such an equilibrized condition is maintained through chemical, how far through finely adjusted mechanical factors, we do not know at present. However, in the somewhat analogous case of the transformation of the connective tissue cells of the uterine mucosa into decidua, we have analyzed the underlying factors experimentally and found that a combination of specific chemical and mechanical factors, which latter are likewise to a certain extent specific, is responsible for this change (6).

In addition, the interstitial cells of the testicle are accessible to that chemical stimulation which is implied in compensatory hypertrophy and they are furthermore affected by lack of common foodstuffs.

2. The specific effects exerted by the interstitial cells have been ascribed by Bouin and Ancel to certain products formed within the cells which microchemically behave like fats or lipoids. We found, in our case, reasons for assuming that drop-like substances were furnished the interstitial cells from the surrounding lymphatics and perhaps also from the blood capillaries. How far the substances taken up from the circulation and perhaps modified within the cells are identical with the substances described by Bouin and Ancel and Whitehead, we cannot at present decide.

3. Various writers, especially Bouin and Ancel, concluded that the interstitial cells of the testicle are responsible for the development of sexual desire in the male. Our observations seem to confirm this view. The complete absence of spermatogonia in the tubules, together with the marked development of the interstitial gland was accompanied by the presence of marked manifestations of sexual instinct.

4. Bouin and Ancel, Steinach (7, 8) and Tandler and Grosz not only ascribe to the interstitial gland the function to cause chemically those nervous changes which find expression as sexual desire, but the additional task of enhancing the development of the male sexual characters, of allowing the full development of prostate, penis, seminal vesicle, and of suppressing the development of the female secondary characters. Our observations apparently contradict these conclusions. In our case, the very marked development of the interstitial gland was accompanied by the absence of penis, seminal vesicles (and presumably of prostate), and on the other hand was associated with a very marked development of the mammary gland. The latter resembled a proliferating breast, a "proliferating gland" such as Dr. Hesselberg and the writer found it in certain stages of the sexual cycle in the female guinea pig (9). Our observations prove that the presence of a very strongly developed interstitial gland is perfectly compatible with the existence of certain female, and with the absence of certain male secondary sexual characters. A priori two interpretations of this fact are possible:

(*a*) The interstitial gland of the testicle has not a specific function in the sense assumed especially by Bouin and Ancel and Steinach. (*b*) The interstitial gland is specifically "male enhancing" in its function, but the end effect does not only depend upon this specific action of the interstitial gland, but also, and primarily so, on the system on which it acts. We have reason for assuming that in a certain respect an individual can be compared to a more or less sensitive balance in his or her sexual potentialities and that different individuals of a certain species differ in the resting point of their sexual equilibrium (10, 11, 12, 13, 14). Without the interference of hormones which are produced by the gonads, the point of equilibrium in some individuals

would be neutral; in others it would be deviating more or less in the direction toward the male; in still others toward the female side. On the whole the male would usually deviate to the male and females to the female side. However, differences exist in this respect in different classes and species of animals and also in individuals of the same species. There are, furthermore, differences in the relative degree of stability of this point of equilibrium. In insects it is apparently very stable and little or not at all influenced by hormones of gonads; in mammals the equilibrium is labile and readily influenced by hormones of the gonads. In mammals, the male hormones can be compared to weights added to the male side of the scales and female hormones influence the scales in the opposite direction. It seems probable that in our case we had to deal with an individual with imperfect male gonads which represented a system with a very slight tendency towards the male side and with a relatively strong tendency toward the female side. In such an individual with tendency toward female secondary sexual characters even a strong male hormone is not able to prevent the growth of the mammary gland, and this female tendency is probably in some way connected with the suppression of male secondary characters during embryonic development, and perhaps also directly or indirectly with the failure of the testicle to descend to its proper place and develop perfectly. In such a relatively stable system tending toward femaleness in its secondary characters, even a specific male hormone, such as is supplied by the interstitial gland of the testicle, has little chance to become effective. Whether, under certain conditions, the hormone given off by the interstitial gland of the testicle might in addition be able to promote growth of the mammary gland, cannot be decided on the strength of the evidence which we have at present.

Summary.

1. It is shown that in a guinea pig with undescended testicle, in which spermatogonia were lacking, and the interstitial gland was hypertrophic, sexual desire was strongly developed.

2. An attempt is made to analyze the various factors that determine the growth of the interstitial gland in the testicle.

3. It is shown that, notwithstanding the presence of a hypertrophic interstitial gland, there was an absence of male secondary sexual characters and that the mammary gland had a female character. A provisional interpretation of these facts is given on the basis of our present knowledge of sex determination.

LITERATURE.

1. **P. Bouin et P. Ancel.**
'03 Archiv. de Zoöl. exper., Série IV., 1903, I., 437, and 1905, III., 390.
2. **Julius Tandler u. Siegfried Grosz.**
'13 Die Biologischen Grundlagen der Sekundären Geschlechtscharaktere. Berlin, 1913.
3. **R. H. Whitehead.**
'08 Anat. Record, 1908, II., 177.
4. **I. Bergonié et L. Tribondeau.**
'05 C. r. Soc. Biol., 1905, LVIII., 155.
5. **G. Herxheimer u. K. F. Hoffman.**
'08 D. Med. Wochensch., 1908, XXXIV., 155.
6. **Leo Loeb.**
'07 Zentralblatt f. allg. Pathol., 1907, XVIII., No. 14.
'08 Journ. Amer. Med. Assoc., 1908, 1897.
'09 Archiv. f. Entwickelungs Mech., 1909, XXVII., 89; 1909, XXVII., 463.
7. **E. Steinach.**
'12 Pflueger's Arch., 1912, CX., LIV., 71.
8. **E. Steinach.**
'13 Zentralblatt f. Physiol., 1913, XXVII., 717.
9. **Leo Loeb and Cora Hesselberg.**
'17 Journ. Exp. Med., 1917, XXV., 285 and 305.
10. **T. H. Morgan.**
'13 Heredity and Sex. New York, 1913.
11. **L. Doncaster.**
'14 The Determination of Sex. Cambridge, 1914.
12. **Jacques Loeb.**
'16 The Organism as a Whole. New York, 1916.
13. **R. Goldschmidt.**
'16 Amer. Naturalist, 1916, L., 705.
14. **F. R. Lillie.**
'17 Proc. Nat. Acad. Sciences, 1917, III., 464.

Additional Note

The foregoing paper was written without the knowledge of two quite recent, very important publications by F. R. Lillie and C. L. Chapin, which may have some bearing on our observations, and which it will therefore be necessary to briefly discuss from

[1] F. R. Lillie, *Journal Exper. Zoölogy*, 1917, XXIII., 371. C. L. Chapin, *ibid.* 453. I am much indebted to Prof. Lillie for calling my attention to these papers,

this point of view. In the first of these two papers Lillie gives a detailed account of the anatomy of the internal and external genital organs in freemartins in various stages of embryonic development and of his interpretation of the cause of the abnormalities and in the second paper Chapin describes the microscopical findings in these cases. It follows from Lillie's observations and analysis that the sterile freemartin represents an individual which was originally a female, but in which under the influence of hormones carried into her circulation from the chorionic vessels of a male twin, the female characteristics were more or less suppressed and male characters acquired. This not only applies to the secondary sexual characters, but even to the structure of the gonads. As Chapin has shown, the development of the ovarian cortex and of ovarian follicles is more or less interfered with or may be altogether suppressed, and the medullary sex cords develop often in a way similar to the seminiferous tubules, although spermatocytes or spermatozoa to not seem to be produced. The development of the Müllerian ducts is likewise interfered with and seminal vesicles may develop instead. The external genitalia and the mammary gland are least affected by the male forming substances, which according to Lillie originated in the testicle of the male twin and were from here transmitted to the female twin; these latter organs remain usually female in character.

These important findings may have some bearing on the interpretation of our observation. There exist undoubtedly marked similarities between the condition found in the freemartins and in the condition of the genital organs of the guinea pig which we examined. However, while Lillie and Chapin examined embryological material, we have to deal with an adult animal.[1] In our case we observed a gonad which shows the characteristics found in testicles under various pathological conditions. Similar changes have been produced in testicles experimentally under the influence of Roentgen rays. The tubules, the character of the cells composing them and particularly of the nuclei closely resemble structures which we found after transplantation of

[1] Microscopic sections through the gonads of an older freemartin from the material of Dr. Lillie show a great similarity with our sections through the gonads of the guinea pig.

testicle and epididymis in the guinea pig. The interstitial cells are developed to an extraordinary extent. The organs were cut in complete serial sections and no trace of ovarian structure was found. From the descriptions given by Miss Chapin, I should judge that a similar condition has not been observed in the fetal gonads of the freemartins. In the guinea pig, uterus and vagina as well as vas deferens, seminal vesicles and penis were absent. While I was unable to find a description of the histology of the mammary gland in freemartins, we observed in our case a typical female mammary gland with the added peculiarity that the mammary glands of both sides were unequal.

The question arises, whether the guinea pig which we observed was an analogue to the freemartin in cattle. Was this animal originally the twin sister of a brother, under whose influence the observed deficiencies were produced? While we cannot definitely exclude such a possibility we must consider the fact that neither ourselves nor anyone else has so far observed twins with a common chorion and deficiencies in the development of the sexual organs in rodents. Lillie himself points out the great improbability of such an occurrence in animals in which the ova enter the uterine wall at an early date and in which the uterine horns are separated. On the other hand, we accidentally observed in several cases absence of one uterine horn in guinea pigs. Malformations of the sexual organs do therefore occur. It is very probable that similar malformations may be due to different interferences in the embryo, just as we know that cyclopic conditions of the eye can be produced in different ways. We interpreted in our paper the conditions which we found in the guinea pig as consisting essentially in the presence of an abnormal, incompletely developed male gonad in which generative cells are absent, and in which the interstitial cells show an unusually pronounced development. This maleness of the gonad and especially the marked development of an interstitial gland is associated with deficiencies in the formation of the efferent genital ducts and with a female mammary gland. We interpreted this unusual condition as due to the original female constitution in the animal, to the tendencies toward femaleness which prevented the male interstitial cells from exerting their specific influences

on the mammary gland, If this guinea pig should represent an analogue to the freemartin in cattle, we would have to assume that the male characteristics of the gonad were produced in an original female through the influence of the testicular substance of the male brother. While such an origin has been made possible through the findings of Lillie, the essential point is that, whatever the origin of this condition, we have every reason to consider the gonad which we described as male.

It is the complete examination of the gonads in serial sections which makes it possible for us to exclude the presence of ovarian structures in this animal and to prove the similarity of the organs with the testicles found under certain experimental and natural pathological conditions. There is no reason for assuming that the gonads in our case represented really ovarian tissue. We have examined many hundred of ovaries of guinea pigs of various ages and have never been able to detect in the ovaries of guinea pigs structures resembling the interstitial gland of the testicle, while on the other hand such structures are strongly developed in testicles which have been retained in the abdominal cavity.

The findings of Lillie and Chapin suggest very strongly, and I take this also to be the interpretation of Lillie, that even in the freemartin the gonad which originally was destined to become female, assumes at least in part true male characters. If this were not so, it would be difficult to explain why seminal vesicles could develop in a freemartin. We should expect that the autogenous ovarian substances would prevent the heterogenous testicular substances from exerting such an influence on the secondary sexual organs of the female twin.

We found in our case a marked development of sexual instinct associated with the absence of generative cells and with a very marked development of the interstitial tissue. Now, as we have pointed out in previous papers, it is improbable that in the guinea pig the interstitial gland is responsible for the manifestation of sexual desire in the female.[1]

[1] Loeb, Leo, *Zentralblatt f. Physiologie*, 1911, XXV., No. 9, and Loeb, Leo, "The Relation of the Ovary to the Uterus and Mammary Gland from the Experimental Aspect." *Surgery, Gynecology and Obstetrics*, 1917, XXV., p. 300. (A review of the literature concerning this and other aspects of the mechanism of the sexual cycle.) In accordance with the most widely accepted view we assumed in our

Typical interstitial cells are absent in the normal ovary of the guinea pig. If we should assume the gonads in this guinea pig to be female our own observation would constitute a decisive proof that also in the female guinea pig the interstitial cells produce the substance which is responsible for the sexual desire—a conclusion which, however, as we stated above, would be contradictory to other facts which we established previously.

The case which we described may be compared to a single equation communicated by nature, in which two variables are present. While one equation is not sufficient to determine the unknown factors in such a case, the variables have at least been carefully defined in our case. We may hope that later a second equation will be found which will permit defining all the variables. We thought it therefore of interest that these observations should be published.

discussion that it is the so-called interstitial gland of the testicle which is responsible for the development of the secondary sexual characters and of sexual desire in the male. We wish, however, to emphasize the fact that in our case epithelial elements were present in the testicle in addition to the interstitial gland and apriori we cannot exclude the possibility that they and not the interstitial gland are the causative agency in determining sexual phenomena. We are likewise not certain that this can be excluded in those experimental and pathological conditions in which the changes were attributed to the interstitial gland. However we are not directly concerned with this question and we are not in a position to contribute definite data which would favor or contradict this view. We wish, however, to reiterate the fact that on various occasions we madé observations which do not seem to accord with the assumption that in the ovary the so-called interstitial gland initiates heat.

PHYSIOLOGICAL SENESCENCE IN HYDROMEDUSÆ.

C. M. CHILD.

The following observations on senescence in the hydromedusæ were made at the Puget Sound Marine Station, Friday Harbor, Wash., during the summer of 1917. I wish to acknowledge my obligation to the director of the laboratory, Dr. Frye, for the use of a room and the privileges of the station, and for his kindness in providing special glassware and other equipment. To other members of the staff I am also deeply indebted for many courtesies.

The great abundance, variety and large size of hydromedusæ at Friday Harbor makes this an extremely favorable locality for work on these forms. During the summer of 1917 five species of hydromedusæ, *Phialidium gregarium*, *Æquorea cærulescens*, *Sarsia rosaria*, *Mitrocoma discoidea* and *Stomatoca atra* could readily be obtained in considerable numbers, often by the hundred, from the laboratory float, and five other species of other genera were more or less abundant, besides two species of siphonophores. The first four species named above furnished the material for the observations and experiments on ageing, and the results are essentially the same in all.

The work was done entirely on free-swimming medusæ and is essentially a comparison of younger and older individuals, no attempt having been made to obtain the medusa-bud stages before detachment from the hydroid.[1] In the selection of individuals of different age for experiment, size of the animal and degree of development of the gonads were used as superficial criteria of age. As might be expected, these two criteria are in general in close agreement, for since the range in variation in size of the medusæ-bud at a given stage of development is not very great, a larger medusa must in general have undergone a greater amount of growth than a smaller, and may therefore be

[1] In *Pennaria* a progressive change in physiological condition has been observed from earlier to later stages of medusa-buds (Child, '15, pp. 150–152).

expected, if age differences exist, to be physiologically older. As has been shown for *Planaria*, and other simple animals (Child, '15, Chapter IV.—VII.) physiological age is not necessarily a function of the length of time which the individual has lived, for nutrition, agamic reproduction and various other factors play a rôle in determining the physiological age at any given time. Size is therefore a far more adequate criterion of age in such forms than time. Moreover, since the development of the gonads in medusæ progresses from a relatively early stage to sexual maturity the condition of the gonads also serves as a measure of the stage in its life history which the animal has reached.

The chief method employed was that of determining the susceptibility of younger and older animals to various agents. The general relation between susceptibility and age has been discussed elsewhere (Child, '14, '15) and need only be briefly restated. In general, in concentrations or intensities sufficiently high to kill without permitting acclimation, the susceptibility decreases with advancing age. In low concentrations, which permit some degree of acclimation, the susceptibility increases with advancing age because the young individual possesses a greater capacity for acclimation then the older, therefore becomes more rapidly and more completely acclimated and so dies later than the older, or lives indefinitely, while the older dies sooner or later.

It has been shown that a relation exists between susceptibility and metabolic activity, more particularly the oxidative or energy-freeing reactions. In general, susceptibility to the higher concentrations or intensities varies directly, and susceptibility to the lower concentrations varies inversely as the rate of these reactions, because acclimation occurs more rapidly and more completely where metabolic activity is greater. This relation between susceptibility and metabolic condition has been shown to hold good not only for different individuals, *e. g.*, young and old (Child, '15) but for different regions of the body of a single individual (Child, '16*a*).

It does not follow, however, from the existence of a relation between susceptibility and rate of metabolism or of certain metabolic reactions, that all agents for which such a relation

exists act directly upon the chemical reactions or that they all act in the same way. Much remains to be learned concerning the nature of the action of external agents on living protoplasm, but the fact is already clear that protoplasmic structure, aggregate condition, permeability, surface tension, etc., are associated with metabolic condition. Susceptibility to a given agent serves at best merely as an indicator of protoplasmic and metabolic condition and tells us nothing concerning the way in which the agent acts. As our knowledge of susceptibility to different agents and conditions increases, we find that under certain conditions different agents give us different results, and such differences in susceptibility relations often serves to throw additional light on the physiological condition of the protoplasm or the action of the agent, or both. The general relations stated above hold for a wide range of agents and conditions, and it is primarily with these general relations that we are concerned in the present paper.

Age-differences in Behavior.

A very conspicuous difference between young and old individuals is the much higher rate of pulsation in the young animal and its progressive decrease with advancing age. Table I. gives characteristic data on this point.

Table I.

Species.	Diameter of Body in Mm.	Pulsations in 20 Ser. at 14° C.
Æquorea cærulescens	7	40
	20	32
	50	20
Mitrocoma discoidea	18	28
	60	16
Sarsia rosaria	5	40
	10	30

In every species observed, ten in all, a similar difference in the rate of pulsation exists, the rate in the sexually mature animal being in most cases about half that of the animal at the beginning or in very early stages of gonad development. By repeated stimulation the rate may often be somewhat increased, particularly in old animals, but the differences in animals under ordinary conditions are of the order of magnitude indicated. This dif-

ference in pulsation rate in itself indicates a very marked decrease in metabolic rate with advancing age. Mayer ('06) has noted that in the scyphomedusa *Cassiopeae* the rate of pulsation decreased with advancing age.

Young and old animals also differ in their behavior in another way. Under the usual conditions periods consisting of a larger or smaller number of pulsations alternate with periods of rest. The total length of pulsation periods as compared with rest periods decreases with advancing age; in other words the younger animal spends a larger portion of the time than the older in rhythmic pulsation. Pulsation periods may either be longer or more frequent or both in the younger animal than in the older. In *Æquorea* this difference is perhaps even more strongly marked than in other forms observed. In the large, sexually mature animals 60–80 mm. in diameter, when under natural and so far as possible constant conditions, the periods of quiescence are often one or two minutes long, sometimes even longer, the animal drifting passively during this time without a single pulsation. Following such a quiescent period a single pulsation or a pulsation period may occur. Under the same conditions the quiescent periods in young animals 15–20 mm. in diameter are usually very much shorter, commonly only a few seconds and the pulsation periods are usually longer than in the old animal. The young animal is then more continuously active and gives the impression of a much greater degree of "spontaneity," since it is usually impossible to distinguish any external exciting factor responsible for the beginning of pulsation after a period of quiescence. In the other species observed similar differences between young and old exist, but periods of quiescence are usually shorter than in *Æquorea*.

The irritability of the young animal as indicated by the effectiveness of direct mechanical stimulation in inducing pulsation during a quiescent period is distinctly greater than that of the old. Usually the quiescent animal in the earliest stages of gonad-development responds by pulsation to the slightest touch in the marginal region, while the sexually mature animals commonly respond only to much more intense mechanical action.

These differences between younger and older medusæ show of

course a considerable range of variation in different individuals, but are nevertheless striking features of the behavior of these animals when attention is directed to them and they remind one irresistibly of age differences in behavior in much higher animals. The apparent restlessness and spontaneity, the more rapid pulsation rate, and the greater irritability of the young animal as compared with the old all suggest that in these, as in other organisms, so for as known, the life history of the individual is from an early stage a process of physiological senescence which expresses itself dynamically as a decrease in rate or intensity of the fundamental energy-liberating metabolic reactions.

Susceptibility.

In determining the susceptibility of the animals various other criteria besides death and disintegration may be used as a check, viz., cessation of rhythmic pulsation, the disappearance of muscular contractility and shrinkage of jelly in some agents. As regards the relation between susceptibility and age all of these criteria give the same results. It is usually difficult or impossible to determine the exact time when an animal ceases to respond to stimulation by rhythmic pulsation or by slow muscular contractions and the early stages of shrinkage and of disintegration are not less difficult to determine. The times given in the following tables represent approximations only. Frequently pulsation or contractility is present at one observation and absent at the next, and the time of its disappearance can only be estimated. Nevertheless there is no difficulty in distinguishing the differences in susceptibility between young and old animals. The experiments were mostly performed with single pairs, one young, one old, but in some cases two or three of each were used.

Æquorea cærulescens.

This, being one of the most abundant species, was used to a large extent. The smallest and youngest individuals found were 8–10 mm. in diameter, without gonads, the largest, and oldest, sexually mature animals 60–75 mm. in diameter. The chief results are tabulated below. In these tables the sign ± following a given time indicates that this is estimated from two observa-

tions between which the change in question occurred, and . . . ? indicates that the change or process did not begin until after the close of the experiment. In this species cessation of pulsation, disintegration and in some cases loss of contractility are available as criteria of susceptibility. The jelly is very firm and does not shrink to any great degree in any agent used.

The three tables agree in showing a greater susceptibility in young than in old animals, as indicated by cessation of rhythmic pulsation, loss of muscular contractility and disintegration. Special attention must be called to certain points. In Table II.

TABLE II.

Æquorea: SUSCEPTIBILITY TO KNC.

Series.		Diameter in Mm.	Conc. KNC.	Duration of Experiment.	No Pulsation after Stimulation.	No Contraction after Stimulation.	Disintegration.
1	*A*..	7	*m*/10000	1 hr.	1 min. ±	5 min. ±	. . .?
	B..	12	After neutral red		5 "	10 " ±	. . .?
	C..	20			10 "	20 "	. . .?
	D..	50			40 "	60 " ±	. . .?
2	*A*..	12	*m*/10000	4 hrs.	30 sec.		. . .?
	B..	30			20 min.		. . .?
I.	*A*..	18	*m*/500	17 hrs.	2–5 min.		12 hrs. ±
	B..	60			10–15 "		17 " ±
II.	*A*..	18	*m*/200	6 hrs.	3 min.		4–6 hrs.
	B..	60			8 "		6–? "
III.	*A*.	16	*m*/100	2 hrs.	At once		1 hr.
	B..	70			" "		2 "
IV.	*A*..	20	*m*/100	3 hrs.	At once		$1\frac{1}{4}$–$1\frac{3}{4}$ hrs.
	B..	60			" "		$1\frac{1}{2}$–3 "

it is of interest to note that KNC *m*/10000 is about as effective, or in some cases more effective than much higher concentrations, *m*/500, *m*/200, in stopping pulsation. It has been observed in other cases (Child, '16*a*, '16*b*) that rapidity of action of KNC and certain other agents, as indicated by death or other effects, increases only slightly with increase in concentration above a

certain limit or may even decrease. The facts suggest that the high concentrations may decrease permeability to themselves, at least up to a certain limit where their action becomes violently destructive to protoplasm, and so practically instantaneous.

In Table III., HCl and Table IV. KOH, the action in stopping pulsation increases very rapidly with increasing concentration,

TABLE III.

Æquorea: SUSCEPTIBILITY TO HCl.

Series.		Diameter in Mm.	Conc. HCl.	Duration of Experiment.	No Pulsation after Stimulation.	No Contraction after Stimulation.	Disintegration.
II.	*A*..	18	*m*/1000	5 days	4½ days	...?	...?
	B..	60			...?	...?	...?
III.	*A*..	15	*m*/1000	5 days	3 days −	4 days +	...?
	B..	40			...?	...?	...?
3 IV.	*A*..	18	*m*/800	5 days	3 days +	4 days ±	...?
	B..	60			...?	...?	...?
V.	*A*..	18	*m*/500	21 hrs.	At once		8–12 hrs.
	B..	60			1 min. ±		17 hrs. ...?
4	*A*..	18	*m*/500	1 hr.	At once		
	B..	60			2 min.		
6	*A*..	18	*m*/400	1 hr.	At once		55 min. ..?
	B..	60			" "		...?
I.	*A*..	18	*m*/300	2¼ hrs.	At once		55 min. ..?
	B..	60			" "		2¼ hrs. ..?
5 II.	*A*..	18	*m*/300	2¼ hrs.	At once		
	B..	60			1 min.		

proportionately much more rapidly than the concentration, because more or less acclimation to the lower concentration occurs. In HCl *m*/1500—not tabulated—acclimation occurs in the young animal to a greater degree than in the old. After five days in this concentration the young animal still shows well-marked rhythmic pulsation after mechanical excitation while

the old animal cannot be induced to pulsate. In concentrations of *m*/1000 or higher, the young animal is always the more susceptible (see Table III.).

The records on the loss of muscular contractility are very incomplete, but it is evident that rhythmic pulsation and general

TABLE IV.

Æquorea: SUSCEPTIBILITY TO KOH.

Series.	Diameter in Mm.	Conc. KOH.	Duration of Experiment.	No Pulsation after Stimulation.	Disintegration.
9 IV. *A*..	16	*m*/1000	84 hrs.	12 hrs. ±	48 hrs. ±
B..	60			48 hrs. ±	...?
8 I. *A*..	18	*m*/800	34 hrs.	10 min.	4–6 hrs.
B..	60			24 hrs.	...?
8 IV. *A*..	15	*m*/800	28½ hrs.	5 min.	3–7 hrs.
B..	60			26 hrs. ±	28½ hrs. ...?
9 III. *A*..	14	*m*/600	9 hrs.	2 min.	4–6 hrs.
B..	60			4–5 min.	9 hrs. ...?
9 II. *A*..	18	*m*/400	9 hrs.	30 sec.	2–4 hrs.
B..	75			1 min. +	3–5 hrs. +
9 I. *A*..	18	*m*/300	5 hrs.	5 sec. ±	1–2 hrs.
B..	70			20 sec. ±	3–5 hrs.
8 II. *A*..	14	*m*/200	5 hrs.	At once	2–4 hrs.
B..	60			10–20 sec.	3–5 hrs.
8 III. *A*..	16	*m*/200	3½ hrs.	At once	1¼–2¼ hrs.
B..	60			20–30 sec.	2–3½ hrs.

muscular contraction are not the same thing. Contractility of the muscles of the umbrella persists in all three agents long after ability to pulsate has disappeared, except in concentrations so high that death is practically instantaneous.

A complete but temporary inhibition of pulsation lasting from a few seconds to several hours occurs in almost all cases in HCl *m*/600. Above *m*/600 inhibition is usually permanent. At

the lower limit of concentration for temporary inhibition only the young member of a pair is inhibited; with somewhat higher concentration both are inhibited for a short time but the young animal usually resumes pulsation before the old if the differences in age are not too extreme. As the concentration increases, the length of the inhibition period increases more rapidly in the young than in the old, until about the upper limit permanent inhibition occurs at once in the young animal while some slight pulsation may reappear sooner or later in the old. In short the age differences in susceptibility appear here as in other features of the action of external agents.

Experiments on recovery after temporary exposure to an agent showed that the relation between recovery and age is the same as in other forms examined. The reappearance of the pulsation response to stimulation is the criterion of recovery used. Where the concentration is not too high, the exposure too long or the difference in age between the animals too great, the young will recover before the old on return to water, but with higher concentrations longer exposures and greater differences in age the old animal recovers earlier and usually more completely than the young. These relations between recovery, age, concentration and period of exposure are of course merely a special case of the age-susceptibility relation. To higher concentrations or longer times of exposure the young animal is more susceptible than the old, but to low concentrations or short times of exposure it is able to adjust itself more rapidly or to recover more rapidly afterward than the old (Child '15, '16*a*). The degree of difference between young and old animals also differs widely with different agents, because acclimation to some agents occurs much more rapidly than to others. The observations on recovery and its limits are as yet only fragmentary and further work is necessary before a complete statement can be made.

Some of the data of an experiment with HCl *m*/500 will serve as an example of recovery. Animals 15 mm. and 60 mm. are placed in HCl *m*/500 and at intervals a pair, one of each size, is returned to water. In the young animals all pulsation ceases at once in HCl, in the old it ceases after two minutes. On return to water after 5–10 minutes in HCl pulsation usually reappears

after 2–3 minutes in both old and young, often slightly earlier in the young. After one hour in HCl pulsation reappears after 10 minutes in the old, and after 12–13 minutes in the young and after 2½ hours in HCl pulsation reappears in the old after 25, in the young after 45 minutes. The longer the exposure the more the young animal falls behind the old in recovery. After 20 minutes in HCl *m*/400 young animals 18–20 mm. did not recover at all while old animals 60 mm. showed slight pulsation after four hours in water. Recovery after KNC is much slower and occurs only in much lower concentrations.

In all concentrations of KOH from *m*/1000 to about *m*/250 a primary acceleration of pulsation rate and increase in strength of contraction occurs, but in concentrations higher than *m*/250 pulsation usually ceases at once or in a few seconds. During this primary acceleration the pulsation rate is often doubled, but since the maximum rate usually persists only a few seconds and is followed by progressive decrease in rate it is difficult to determine whether the degree of primary acceleration differs in a characteristic way with age. It is certain, however, that the retardation following the acceleration occurs more rapidly in young than in old animals, so that pulsation ceases first in the young, as Table IV. indicates.

The opposite primary effects of acid and alkali on the pulsation rate are what might be expected in the light of what we know of their action on living protoplasm in general. In lethal concentrations, however, their action, following the primary effect, is essentially similar and the relations between susceptibility and age are the same for both.

Mitrocoma discoidea.

In this species the jelly is much less firm than in *Æquorea*, and marked shrinkage occurs in the young animals in acids and some other agents. Muscular contraction as distinguished from pulsation is much more frequent even in nature than in *Æquorea*. Frequently contraction brings about a folding of the umbrella with the two halves of the margin approximated or apposed along a straight line, or the margin may become square or very irregular in outline. The contracted condition may persist for several

minutes, or after very strong stimulation an hour or two before return to the usual form and resumption of pulsation occur. As in *Æquorea*, the ability to contract persists long after the ability to pulsate has disappeared.

In Table V. the chief data obtained with HCl are given. They are essentially similar to those for *Æquorea*.

TABLE V.

Mitrocoma: SUSCEPTIBILITY TO HCl.

Series.		Diameter in Mm.	Conc. HCl.	Duration of Experiment.	No Pulsation after Stimulation.	No Contraction after Stimulation.	Beginning of Shrinkage.	Disintegration.
X.	*A*	18	*m*/1500	114 hrs.	...?	Acclimated: active at end of expt.		
	B	60			114 hrs. −	...?	...?	...?
II.	*A*	18	*m*/1000	55½ hrs.	40 hrs. ±	...?	...?	...?
	B	60			...?	...?	...?	...?
III.	*A*	18	*m*/800	48 hrs.	8½ hrs.	24 hrs. +	24 hrs.	40 hrs. ...?
	B	55			40 hrs. ±	...?	...?	...?
IV.	*A*	15	*m*/700	48 hrs.	At once	24 hrs. ±	30 hrs. ±	40 hrs. ...?
	B	60			15 hrs.	40 hrs. ±	...?	...?
V.	*A*	20	*m*/650	34 hrs.	2 hrs.			30 hrs. ...?
	B	45			15 hrs. ±			34 hrs. ...?
VI.	*A*	18	*m*/650	52½ hrs.	26 hrs. ±	24 hrs. ±	22 hrs.	45–52½ hrs.
	B	60			34 hrs. ±	40 hrs. ±	...?	50 hrs. ...?
VII.	*A*	18	*m*/600	51½ hrs.	At once	24 hrs. ±	32 hrs.	45–51½ hrs.
	B	60			26 hrs. ±	...?	...?	...?
VIII.	*A*	18	*m*/500	4½ hrs.	At once	1 hr. ±	2 hrs.	
	B	60			" "	...?	...?	
IX.	*A*	20	*m*/500	9¾ hrs.	" "	At once		
	B	60			" "	...?		

The first series 5, X. in HCl *m*/1500 shows acclimation as regards the effect on pulsation rate, pulsation persisting longer in the young than in the old. In *Æquorea* also some degree of acclimation usually occurs in this concentration (p. 53). The

long persistence of pulsation in both members of the pair in Series 5, VI. (HCl $m/650$) as compared with the other series is anomalous and no reason for it is known. Otherwise the data in the table are consistent and show that for the concentrations included, except $m/1500$, the young animal is more susceptible than the old.

Results with KNC show similar relations between age and susceptibility, of course with much lower concentrations. The results of one series on pulsation susceptibility are given in Table VI.

TABLE VI.

Mitrocoma: SUSCEPTIBILITY OF PULSATION TO KNC.

Series.	Diameter in Mm.	Conc. KNC.	Duration of Experiment.	Pulsation.
4. IV. *A*..	18	$m/100000$	5 hrs.	Pulsation only retarded: rate $A = \frac{1}{2}$ rate B.
4. IV. *B*..	60			
4. III. *A*..	18	$m/50000$	5½ hrs.	Very slight, irregular.
4. III. *B*..	60			Well-marked, regular.
4. I. *A*..	16	$m/25000$	½ hr.	Ceases in 10–15 min.
4. I. *B*..	60			" " 15–20 "
4. V. *A*..	18	$m/10000$	½ hr.	Ceases in 5 min. ±
4. V. *B*..	55			" " 15 " ±

In the first pair, 4, IV., $m/100000$, the susceptibility is shown, not in the time of cessation of pulsation, but in the retardation of the rate. At the beginning of the experiment rate $A = 2$, rate B approximately, but after five hours rate $A = \frac{1}{2}$ rate B. In the second pair, 4, III., $m/50000$, pulsation in the young animal is almost completely inhibited after 5½ hours, while in the old it is still strong. Here rate A was very much lower than rate B. In the higher concentrations pulsation ceases in a few minutes.

A few experiments with other agents give similar results. In neutral red animals 18–20 mm. long cease to pulsate, undergo shrinkage and begin to disintegrate much earlier than animals 60 mm., although no marked difference in rate or intensity of staining is apparent. In methylene blue 1/30000, increased to

1/15000 after 24 hours, the animals live for several days with all cellular tissues stained. After 48 hours pulsation rate $A = \frac{1}{2}$ pulsation rate B approximately, where A and B are animals of 18–20 and 50–60 mm. respectively.

Phialidium gregarium.

The youngest animals found were mostly 7–8 mm. in diameter, while the old, sexually mature individuals measured 20–25 mm. Only a few series of susceptibility experiments were made with this species, HCl *m*/1500, *m*/1000, *m*/800, *m*/600, neutral red and methylene blue being used. The susceptibility relations are similar to those observed in the other species. In this rather delicate form shrinkage is a characteristic feature of the action of various agents, the younger animals undergoing shrinkage earlier and to a greater degree than the old, a decrease of half or three fourths in diameter occurring in many cases.

Sarsia rosaria.

In this species the umbrella is very deep, the oral-aboral axis being almost or quite twice the diameter. Depth is therefore a more convenient measure of size than diameter and is commonly used. The sexually mature animals average about 15 mm. in depth of umbrella, but the extremes range from 10–12 mm. on the one hand to 20 mm. on the other. In the smallest and youngest animals found, 6–7 mm. in depth, the gonads had already begun to develop, and although the susceptibility of these forms is characteristically greater than that of the mature individuals the differences in age and in susceptibility in the animals available are not as great as in the other species examined.

In general, results with this species are similar to those with others but in some pairs an individual 12–15 mm. showed a susceptibility equal to or even greater than that of 7–8 mm. animals. Since none of the animals within these limits of size represent very great differences in physiological age, all being near sexual maturity, it is to be expected that occasionally a larger animal will show a higher susceptibility than a smaller.

The agents used were KNC *m*/50000, *m*/10000, *m*/5000, HCl *m*/1000, *m*/600, *m*/500, *m*/400, neutral red and methylene blue,

and the age differences appeared in all with the few exceptions above noted. One of these exceptions was rather remarkable. In KNC *m*/50000 animals 7–8 mm. usually lose the ability to pulsate, even on stimulation, in 3–10 minutes, animals 15 mm. after 1–2 hours, while in *m*/10000 the times for cessation of pulsation are respectively 1–2 minutes and 3–10 minutes. In one case, however, in KNC *m*/10000 an animal 15 mm. ceased to pulsate in three minutes, while an animal 8 mm. in the same closed container continued normal pulsation during thirty minutes, longer observation being impossible. For some reason this particular animal was practically insusceptible to KNC *m*/10000, a concentration which stops pulsation in the younger individuals of the other species examined in 5 minutes or less and in other younger individuals of *Sarsia* in 1–3 minutes. This extreme exception is merely recorded without any attempt to account for it. The observations on *Sarsia* do indicate a wider range of physiological condition in animals of a given size than in the other species examined, but they afford no clue to interpretation of a condition so extremely exceptional as this.

Conclusion.

The preceding data make it evident that the hydromedusa, like other animals, undergoes a progressive change in physiological condition with advancing development, the differences in behavior and susceptibility being indicators of this change. It has been shown that in other forms a decrease in the rate of oxidations is a characteristic feature of the change and the facts indicate that a similar decrease occurs here, although these forms are not very favorable material for the direct determination of oxygen consumption or CO_2-production. The assumption that the differences in susceptibility are due merely to differences in permeability of surface membranes is refuted by the results obtained with neutral red and methylene blue and by the cases of acclimation. Moreover, there is every reason to believe that the age differences in susceptibility to cyanides are at least more directly associated with the oxidation rate than with permeability.

As regards permeability, however, it is becoming more and more evident to investigators in this field that the condition of

the membrane is not independent of the metabolic condition of the protoplasm. Permeability to various agents is itself within certain limits an indicator of age or physiological condition of the cell.

The progressive change in behavior, the decrease in pulsation-rate and "spontaneity" indicate very clearly a process of physiological senescence and the data on susceptibility only confirm and extend the facts of observation.

Summary.

Four species of hydromedusæ show evidence of a process of physiological sensecence in the decrease in pulsation-rate and apparent spontaneity and in the decrease in susceptibility to KNC, HCl, KOH and "vital" dyes in various concentrations with advancing development. The criteria of susceptibility are change in rate or cessation of pulsation, loss of contractility, shrinkage of the jelly and disintegration of cellular tissues, and all these criteria agree. The facts justify the conclusion that a decrease in the rate of metabolism, or more specifically of oxidations, is a characteristic feature of this process of senescence.

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References.

Child, C. M.

'14 Starvation, Rejuvenescence and Acclimation in *Planaria dorotocephala*. Arch. f. Entwickelungsmech., XXXVIII.

'15 Senescence and Rejuvenescence. Chicago.

'16a Experimental Control and Modification of Larval Development in the Sea Urchin in Relation to the Axial Gradients. Jour. Morphol., XXVIII.

'16b Further Observations on Axial Susceptibility Gradients in Certain Algæ. Biol. Bull., XXXI.

Mayer, A. G.

'06 Rhythmical Pulsation in Scyphomedusæ. Carnegie Inst. Publ. No. 47.